The phenotypic correlates of individual vulnerability to angling

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I. Silva Uusi-Heikkilä, Christian Wolter, Thomas Klefoth, Robert Arlinghaus, 2008. A behavioral perspective on fishing-induced evolution. Trends in Ecology & Evolution, 23:419-421.

II. Thomas Klefoth, Christian Skov, Jens Krause, Robert Arlinghaus, 2012. The role of ecological context and predation risk-stimuli in revealing the true picture about the genetic basis of boldness evolution in fish. Behavioral Ecology and Sociobiology, 66:547-559.

III. Thomas Klefoth, Christian Skov, Kim Aarestrup, Robert Arlinghaus, 2013. Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*). Fisheries Research, 146:18-26.

IV. Thomas Klefoth, Philip Rebensburg, Christian Skov, Anna Kuparinen, Robert Arlinghaus, unpublished. Towards a mechanistic understanding of vulnerability to hook-and-line fishing – the role of behavior as a basis for understanding angling-induced evolution. Submitted to Evolutionary Applications (05.09.2015) and invited for resubmission.

V. Jun-ichi Tsuboi, Kentaro Morita, Thomas Klefoth, Shinsuke Endou, Robert Arlinghaus, 2016. Behaviour-mediated alteration of positively size-dependent vulnerability to angling in response to historical fishing pressure in a freshwater salmonid. Canadian Journal of Fisheries and Aquatic Sciences, 73:461-468.

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X. Robert Arlinghaus, Kate Laskowski, Josep Alós, Thomas Klefoth, Christopher T. Monk, Shinnosuke Nakayama, Arne Schröder, 2016. Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish and Fisheries, 2016.

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Abstract (english)

Humans have fished at all times and fishing activities have always been selective for certain species, traits and phenotypic expressions. These nonrandom fishing activities can lead to human-induced evolutionary changes. The potential for fishing-induced evolution has been intensively discussed in recent years, but most studies have focused on life-history traits that directly or indirectly determine body size in the context of commercial fisheries. Much less is known about potential evolutionary changes in the context of passive angling fisheries. Using comprehensive phenotypic descriptions covering several behavioral, life-history, morphological, and physiological traits, I disentangled the phenotypic correlates of individual vulnerability to angling gear. Using both, benthivorous and piscivorous model species I identified the strength and direction of selection. I then compared survival and reproductive fitness of vulnerable and invulnerable individuals to predict the evolutionary potential of angling-induced selection. My research showed that boldness in the context of foraging is the most important trait under selection in passive fisheries targeting benthivorous species whereas aggression determines selection in piscivorous species. In addition, growth and boldness were positively correlated. Intrinsically high foraging activity- and speed likely explained why explicitly bold fish were caught more often even if several fish were within close vicinity of the baited hook. These highly vulnerable individuals also faced higher natural mortality at the juvenile stage in ponds and within a 25 ha natural lake. Thus, angling-induced selection and natural selection point into the same direction at the juvenile stage. However, using adult, nest-guarding fish, I showed that angling-induced selection can severely impact reproductive fitness when behavioral patterns that determined fitness, like aggression, also affect their vulnerability to angling gear. Based on similarities in life-history and behavior of many benthivorous and piscivorous fish, these results can likely be transferred to many other species. As a consequence of my findings, an exploitation-induced timidity syndrome can be assumed in highly exploited fish stocks leading to increasing shyness and an overall reduced vulnerability of the individual fish. This will have negative consequences for the number of trophy fish, management decision making in the presence of hyperdepletion, and angler satisfaction in general. From a management perspective my findings call for a promotion of behavioral diversity which might be achieved through intensified release of large, bold, and fast growing individuals.

Zusammenfassung (deutsch)

Die Fischerei ist Teil der menschlichen Kultur und selektiv gegenüber bestimmten Merkmalen der Fische, wodurch eine menschlich-induzierte Evolution hervorgerufen werden kann. Das Potential evolutionärer Veränderungen von lebensgeschichtlichen Merkmalen durch kommerzielle Fischerei fand in den vergangenen Jahren große Beachtung, wohingegen das evolutionäre Potential selektiver anglerischer Fischentnahme kaum berücksichtigt wurde. Durch intensive Beschreibung individueller Merkmale wie Verhalten, Lebensgeschichte, Morphologie und Physiologie der Fische habe ich die phänotypischen Korrelate der individuellen Angelfangbarkeit entflechtet. Anhand benthivorer und piscivorer Modellarten konnte ich so die Stärke und die Richtung anglerischer Selektion bestimmen. Zudem habe ich die Überlebenswahrscheinlichkeit und den Reproduktionserfolg dieser Fische gemessen, um das evolutionäre Potential anglerischer Fischereisterblichkeit abschätzen zu können. Ich konnte zeigen, dass die Risikofreude im Zusammenhang mit der Nahrungsaufnahme bei benthivoren Fischen dem stärksten Selektionsdruck ausgesetzt ist, während bei piscivoren Arten Aggression die bestimmende Eigenschaft war. Zudem waren Risikofreude und Wachstum positiv korreliert. Die intrinsische Fraßaktivität- und Geschwindigkeit erklärte, warum risikofreudige Fische selbst in Gruppen die höchste Hakwahrscheinlichkeit aufwiesen. Diese besonders leicht fangbaren Individuen wurden zudem mit höherer Wahrscheinlichkeit von Räubern in Teichen und in einem 25 ha großen Natursee gefressen, sodass anglerisch induzierte und natürliche Selektion bei juvenilen Fischen in die gleiche Richtung wiesen. Bei adulten, nestbewachenden Fischen konnte ich zudem zeigen, dass Eigenschaften, die zu einer erhöhten Fangbarkeit führen, auch den Laicherfolg steigern, sodass anglerische Selektion negative Auswirkungen auf den Reproduktionserfolg haben kann. Diese Resultate können mit hoher Wahrscheinlichkeit auf viele Arten übertragen werden. Folglich muss in stark beangelten Fischbeständen von einem anglerisch induzierten Schüchternheits-syndrom ausgegangen werden, wodurch die Anzahl großer, risikofreudiger und aggressiver Fische sowie die Fangraten und die Zufriedenheit der Angler sinken und die Bewirtschaftung erschwert wird, da die Fangraten von der Fischbestandsdichte entkoppelt werden. Meine Ergebnisse deuten auf eine hohe Schutzwürdigkeit individueller Verhaltensdiversität hin, welche am leichtesten durch den Schutz besonders großer, schnellwüchsiger und risikofreudiger Fische erreicht werden kann.

Background

Humans have fished since the origin of our species and fishing activities are almost always nonrandom in the animals which are selected for harvest (Law 2000). High exploitation rates, nonrandom fishing activities or random fishing activities with high mortality rates can result in overexploited fish stocks and human-induced evolutionary changes (Rijnsdorp 1993; Law 2000; Swain et al. 2007; Heino et al. 2015). A key factor regulating the sustainability of fisheries is the dynamics that determine catchability, i.e. the relationship between fishing mortality and resource abundance. Unfortunately, this relationship is often poorly understood and rarely quantified (Arreguín-Sánchez 1996). Traditionally, catchability has been defined as the fraction of the fish population that is potentially vulnerable to the gear in use. However, individual fish differ in their phenotypic traits and this approach omits individual differences in vulnerability to capture, and as a result potentially overlooks many processes that determine catchability. Thus, catchability should not be seen from the perspective of the whole population, but should instead be considered from the perspective of individual fish to better understand the potential causes and consequences of fishing activities (Maunder et al. 2006). Consequently, the study of how individual phenotypic traits determine vulnerability to fishing gear, determine the probability of capture and, as a result, determine selection, is crucial to further our understanding of fisheries dynamics, stock assessments, and human-induced selection.

The potential for fishing-induced evolution has been intensively discussed in recent years, but most studies have focused on life-history traits in the context of commercial fisheries that directly or indirectly determine body size (Kuparinen and Merilä 2007; Jørgensen et al. 2007). Under the common scenario of size-selective harvest of bigger fish, large fish face a fitness disadvantage that might cause rapid evolution towards earlier maturation at smaller sizes, higher reproductive investment, lower intrinsic growth capacity and, collectively, smaller size at age (Jørgensen et al. 2007). However, many studies overlooked the selection pathways that drive the observed life-history changes. For example, evolution of small body size can result from direct selection against growth rate, but could also be a result of direct selection against correlated behavioral traits (Biro and Post 2008; Enberg et al. 2012) and even less is known about these selection pathways in passive angling fisheries. The potential for evolution of behavioral, physiological or morphological traits and its consequences for life-history, demography and fishing quality in angling fisheries are poorly understood and need deeper investigation which is the main motivation of this thesis.

1. The mechanistic basis of individual vulnerability to passive gear

1.1 Morphology

Most passive fisheries typically operate positively size-selective (Rudstam et al. 1984; Miranda and Dorr 2000; Hubert et al. 2012; Olsen et al. 2012). This is based on managerial reasons due to the application of size-based harvest limits (Garcia et al. 2012), gear specifications in use (Hubert et al. 2012), and morphological characteristics of larger fish (Lewin et al. 2006). In passive recreational fisheries like angling, selective harvest of larger fish is often related to the size of the baits and lures (Lewin et al. 2006; Arlinghaus et al. 2008a) and ingestion of a baited hook was shown to be positively correlated with the size of the fish in longline fisheries (Kaimmer 1999). These findings can be linked to morphological characteristics of large individuals, because an increasing gape size facilitates ingestion of the hook (Lewin et al. 2006; Alós et al. 2008).

McClanaham and Mangi (2004) compared the selectivity of small- and large traps, hand-lines, gill-nets, beach seines and spearfishing under tropical conditions in a marine environment. They reported that hand-line fishing showed the most distinct selection in terms of high trophic level species and particularly large individuals. Similarly, Beukema and De Vos (1974) reported the larger-sized portion of a freshwater carp population in replicated ponds to be 20-30 % more likely to be captured by angling than their smaller-than-average counterparts from the same water bodies, and Rapp et al. (2008) showed that smaller hooks catch more and larger fish in specialized carp fisheries. While higher catch rates of larger individuals in larger traps or nets with large mesh sizes are simply based on physical reasons, the high potential for size-selectivity in hand-line fishing and angling is less intuitive. However, larger fish generally show higher swimming speeds (Stamps 2007), are often dominant (Jenkins 1969), often have larger home ranges (Nash et al. 2015), have higher reactive distances towards prey (Breck and Gitter 1983), might be more experienced at risk-avoidance (Rapp et al. 2008), and are characterized by larger consumptive demands compared to smaller fish (Clarke and Johnston 1999; Mittelbach et al. 2014), cumulatively leading to intrinsically higher vulnerability of large compared to small fish.

Using geometric morphometrics, Alós et al. (2014) found evidence that, also independent of body-size, fishing mortality acts selective for body shape in two commonly targeted coastal fish species. Individuals with larger mouths, shallower and more elongated bodies of the population were found to be more vulnerable to angling. Selection against shallower and more elongated bodies was interpreted as an indirect selection for behavioral traits that co-vary with morphological metrics (Alós et al. 2014) so that shallow bodied individuals might be more active, thereby increasing the encounter probability with the fishing gear, similar to characteristics of highly vulnerable large individuals. Therefore, phenotypic traits like behavior, cognition, learning, physiological demands and energetic state, which are often correlated with size and other morphological characteristics, likely directly impact the vulnerability to angling gear and other passive fishing tactics.

1.2 Behavior

Passive fishing gear such as hook-and-line, gill-nets and traps are known to be selective for certain behavioral expressions of the fish (Hayes 1989; Heino and Godø 2002; Biro and Post 2008; Phillip et al. 2009; Enberg et al. 2012; Hubert et al. 2012; Olsen et al. 2012; Diaz Pauli et al. 2015). The success of passively operated fishing gear depends on the active decision of the individual fish to encounter the gear and to swim into a gill-net or a trap or to ingest a baited hook (Alós et al. 2012; Hubert et al. 2012). In particular energy-acquisition related behaviors are likely to play a fundamental role in determining vulnerability because the probability of capture is strongly related to the amount and location of food ingestion as well as the propensity to find, approach, attack or ingest a lure or bait. As a consequence, evolutionary changes in adult growth rate reported in response to intensive recreational angling (e.g., Saura et al. 2010) might be an indirect consequence of direct selection acting on resource-acquisition-related behaviors (sensing and finding food, searching for food, feeding in the presence of a predator; Biro and Post 2008; Enberg et al. 2012). One of the key resource-acquisition behaviors in fishes is boldness. Boldness is defined as an individual`s reaction to any risky, but not novel situation (Réale et al.

2007), and there is growing evidence of a positive correlation between boldness-related traits like exploration behavior, aggression or risk-taking with vulnerability to angling tactics (Cooke et al. 2007; Härkönen et al. 2014; 2016; Alós et al. 2015a). Similar results have also been reported for other passive gears like traps, beach seines or gill nets (Wohlfarth et al. 1975; Wilson et al. 1993; Biro and Post 2008; Biro and Sampson 2015). These findings are clearly supported by theoretical arguments and simulation models (Alós et al. 2012; Enberg et al. 2012), but experimental evidence for the linkage between behavioral traits and vulnerability to angling is still scarce, especially in the wild, although there has been an increasing number of studies on this topic in recent years (Redpath et al. 2009; 2010; Wilson et al. 2011; 2015; Alós et al. 2014; 2015a; Härkönen et al. 2014; 2016; Kekäläinen et al. 2014; Vainikka et al. 2016), of which, the majority support a behavioral basis of vulnerability to angling.

Using an individual-based modelling approach, Alós et al. (2012) showed that passive angling fisheries can strongly select against high movement rates and intense exploration behavior. Such trait-selective fisheries would leave behind generally more timid and less explorative individuals that are very difficult to catch. These findings were recently confirmed by empirical field observations where harvesting by passive fisheries techniques like gill-netting, trapping and hook-and-line fishing selected against active vertical migration behavior in acoustically tagged cod (*Gadus morhua*), but not against home-range size and horizontal migration (Olsen et al. 2012). In other taxa like pheasants (*Phasianus colchicus*), lobster (*Homarus gammarus*), and elk (*Cervus elaphus*), vulnerability towards hunting or trapping increased with increasing boldness, activity and fast behavior whereas shyness and particularly small home-ranges during the time of hunting and trapping increased survival probability (Ciuti et al. 2012; Wiig et al. 2013; Madden and Whiteside 2014). Similarly for angling, Alós et al. (2015a) found carnivorous painted comber (*Serranus scriba*) to behave significantly more timidly towards baited hooks on high-pressure fishing sites compared to low-pressure fishing sites, but could not detect such differences in behavior for the omnivorous annular seabream (*Diplodus annularis*), indicating a potential species-specific response of behavioral alterations as a consequence of harvesting selection.

Recently, selective pressure through angling on risk-taking related behaviors was also detected from measurements under more standardized conditions within the laboratory and ponds. Härkönen et al. (2014) found activity of brown trout (*Salmo trutta*) to be predictive for their vulnerability towards artificial fly-fishing techniques, and Härkönen et al. (2016) observed that Eurasian perch captured on artificial lures were more active and explorative than their counterparts caught on natural bait. However, no correlation between boldness-related behaviors and angling vulnerability could be observed for the same species when angling was conducted with natural baits alone (Vainikka et al. 2016). Similarly, Kekäläinen et al. (2014) found no differences in purely laboratory assessed boldness-related traits between Eurasian perch that were caught by ice-fishing using artificial- and natural baits. Thus, fisheries-induced selection on behavior might not only be species-specific, but might also rely on an interaction between species, baits, and fishing techniques used (Wilson et al. 2015). Additionally, the feeding ecology of the species of interest may be important (Stoner 2004). It is likely that aggression plays a dominant role in piscivorous species (Biro and Post 2008; Philipp et al. 2015), whereas behaviors related to energy acquisition might be more predictive for the vulnerability of omnivorous fish (Alós et al. 2015a).

In addition to energy-acquisition, aggression, boldness in general, and the likelihood of finding a baited hook, individual behavior of the fish, once the bait is located, might also contribute to individual differences in angling-vulnerability. Fish in close contact with a baited hook can display a number of behaviors with different intensities. Based on certain stimuli (visual, taste and olfactory, and mechanical perceptions), the fish may categorize the baited hook as either edible or inedible and respond accordingly (Kaimmer 1999; Løkkeborg et al. 2010), although these behaviors might be again correlated with general boldness. Fernö and Huse (1983) distinguished ten different behaviors that vary in how a baited hook is approached and ingested that have been modified by Løkkeborg et al. (2010). These behaviors might intrinsically differ between individuals leading to differences in their individual vulnerability once they are in close contact to the baited hook. For example, observations from imaging sonar suggest only a small fraction of sablefish and pacific halibut attracted to longlines were captured (Rose et al. 2005), only one third of halibut interactions with the hook resulted in a bite (Kaimmer 1999), and out of 1252 cod in the vicinity of 160 baited hooks, only 62 individuals were caught on natural bait (He 1996). Similarly, only between 11 % and 37 % of bites from haddock and cod resulted in a capture event, although these fish made up to 35 behaviors towards the baited hooks (Løkkeborg et al. 1989), and out of 95 European catfish, 80 % were interested in a baited hook but only 12.5 % of all fish got hooked (Boulêtreau et al. 2016). However, individual behaviors towards the baited hook are also strongly influenced by learning from past experience (Løkkeborg 1990; Askey et al. 2006), and potentially also from social learning (Beukema 1969; Raat 1985; van Poorten and Post 2005) and trade-off decision making between accuracy and speed of learning that might additionally differ between proactive and reactive individuals (Sih and Del Guidice 2012). Decreasing vulnerability as a consequence of individual and/or social based learning in a catch-and-release context has been shown for both omnivorous (Beukema 1969; Beukema and de Vos 1974; Raat 1985) and piscivorous species (Anderson and LeRoy Heman 1969; Beukema 1970; Hackney and Linkous 1978; O'Grady and Huges 1980, van Poorten and Post 2005; Askey et al. 2006), and can be seen as a plastic response, because it does not involve selective removal other than unwanted hooking mortality. However, the ability to learn also has a genetic basis (Huntingford and Wright 1992), carrying the potential for evolutionary changes through selective harvest. Thus, individual behavioral differences in the vicinity of the baited hook and individual and social-based learning in combination with aggression, boldness- and energy acquisition related behaviors potentially determine hooking probabilities.

1.3 Physiology

Physiology of fishes is the scientific study on how the component parts of the organism operate together (Prosser 1991). Important parts of fish physiology are energy metabolism and metabolic rate. Intrinsic oxygen demands and metabolic rate vary consistently between individual fish and can impact their personality and performance (Careau et al. 2008; Biro and Stamps 2010; Careau and Garland 2012), likely also influencing their vulnerability towards angling gear over a complete life-time (Cooke et al. 2007; Redpath et al. 2009; 2010, Philipp et al. 2015). High energy and oxygen demands have been shown to co-vary with boldness and exploration (Jenjan et al. 2013), traits known to be correlated with learning abilities and proactive life-styles in different fish species (e.g. rainbow trout *Oncorhynchus mykiss*, Sneddon 2003; three-spined sticklebacks *Gasterosteus aculeatus*, Tudorache et al. 2007; carp *Cyprinus carpio*, Mesquita et al. 2015). These integrated phenotypes (Murren 2012) have a metabolic basis (Careau et al. 2008) that impacts the integrated phenotypic trait “vulnerability to angling” (Redpath et al. 2010).

Physiological, behavioral, and life-history traits can be integrated along a pace-of-life syndrome from “slow” to “fast” (Réale et al. 2010). The pace-of-life syndrome hypothesis suggests that closely related species should differ in a suite of physiological traits that have coevolved with the life-history particularities of each species (Ricklefs and Wikelski 2002; Réale et al. 2010). This hypothesis can also be applied to the study of covariation among traits between individuals from the same population (Réale et al. 2010). For example, consistent individual differences in metabolic rate can affect the behavioral output and the life-history productivity of individuals (Biro and Stamps 2010) resulting in the metabolic rate and boldness-related expressions of the fish being positively linked (Cutts et al. 1998; Killen et al. 2012; Myles-Gonzalez et al. 2015). In a fisheries context, activity and boldness were found to be related to growth in rainbow trout (Biro et al. 2004), and individuals with a fast life-style, bold behavior, and high growth rate were found to be more vulnerable to fishing gear (Biro and Post 2008). Consequently, selection against behavioral traits can also result in selection against fast growth and high metabolic rates. The pace-of-life syndrome theorem builds a framework to consider the impact of correlated trait selection with metabolic rate as a basis also in a fisheries context (Mittelbach et al. 2014).

Individuals with a high standard metabolic rate increase their maximal metabolic rate to maintain energetic scope (Priede 1985). Metabolic scope, i.e. the maximal difference between standard metabolic rate and active metabolic rate, might impact vulnerability to fishing, because it is known to influence aerobic activity (Priede 1985), recovery (Killen et al. 2007), and foraging activity (Fu et al. 2009) that together can impact foraging success and energy acquisition behaviors. This relationship again corresponds with the pace-of-life syndrome theory, becausea need to increase maximum oxygen demands necessarily impacts energy acquisition and risk-taking, with measurable fitness consequences for the fish (Biro et al. 2004).

When comparing two strains of largemouth bass selected for their vulnerability to angling, Cooke et al. (2007) found resting cardiac activities (an indirect measure of metabolic rate) to be significantly elevated in highly vulnerable fish. Similarly, Redpath et al. (2010) found standard metabolic rate, maximal metabolic rate, and metabolic scope to be significantly lower among largemouth bass with low angling vulnerability. These studies collectively indicate high metabolic costs for fish that are vulnerable to fishing (Philipp et al. 2015) such that highly vulnerable fish should consume more food (Cooke et al. 2007; Redpath et al. 2009). These differences in energetic demands can impact foraging behavior, energy conversion, and feeding urgency (Nannini et al. 2011), ultimately influencing their vulnerability to fishing gear.

All fish transition from states of postprandial rest to states of physiological arousal when they are hungry (Pavlov 1962), and these transient physiological states influence vulnerability of fish in the short-term (Redpath et al. 2009; 2010). Among the factors that motivate fish to strike baits, including aggression, social facilitation, and curiosity, hunger has the greatest influence on fish vulnerability (Stoner 2003; 2004). Hunger stimulates food search behaviour and reduces risk-avoidance behaviours (Godin and Crossman 1994; Heermann et al. 2013; Løkkeborg et al. 2014; Atema 1980). Accordingly, the response to baited hooks by fish differs as a function of their recent food consumption (Løkkeborg et al. 1995; Stoner 2003; Stoner and Sturm 2004) and food searching behavior increases with food deprivation (e.g. Løkkeborg et al. 1995; Stoner 2003; Stoner and Sturm 2004). These motivations to feed also influence hook ingestion, and food deprived whiting (*Merlangius merlangus*) and cod (*Gadus morhua*) swallowed a hook more rapidly and were more often hooked in the stomach (Fernö et al. 1986 and citations therein). It follows that fish that are satiated or near satiated become more selective of prey items whereas fish that are hungry are easier to capture affecting individual vulnerability (Olla et al. 1970). Thus, physiological demands like metabolic rate and physiological status like hunger can strongly influence the vulnerability of fish towards fishing gear, albeit more research on this topic is needed (Cooke et al. 2009; Killen et al. 2015).

1.4 Life-history

Many studies take the approach that (commercial) fisheries can be seen as a “large-scale experiment on life-history evolution” (Rijnsdorp 1993; Law 2000; Swain et al. 2007; Heino et al. 2015). This is because fisheries usually select for large body sizes and increase adult mortality (Law 2000; Heino and Godø 2002), leaving behind smaller and earlier maturing individuals with decreased fecundity (Olsen et al. 2004). These changes in life-history traits are usually not based on trait-selective harvest, because evolutionary changes in the size and age at maturation primarily emerge from unselective harvesting when mortality in the adult life stages is high (Lennox et al. unpublished). However, also for angling fisheries the potential to induce life-history changes in an evolutionary context exists (Arlinghaus et al. 2009; Saura et al. 2010; Alós et al. 2014) and in this context evolutionary changes of life-history parameters are usually based on trait-selective mortalities that indirectly impact life-history parameters (Redpath et al. 2010; Nannini et al. 2011; Philipp et al. 2015). One life-history trait that often indirectly links vulnerability to capture is growth rate. Growth-rate might be the true basis of life-history changes through passive fishing activities, because the fastest growing individuals are usually harvested first due to their increased willingness to ingest gear and their increased encounter probability with fishing gear (Alós et al. 2012; 2014). Thus, indirect selection on life-history traits might have a physiological and/or a behavioral basis.

Obviously, key processes involved in energy budgeting are behavior-based energy acquisition, surplus energy allocation and somatic growth (Enberg et al. 2012). High energy acquisition caused by intensive foraging will foster fast somatic growth rates in juvenile fish (Heino et al. 2008; Lester et al. 2014). If a behavioral mechanism for selection on life-history traits exists, selection on growth rate as a consequence of size-selective removal should be distinguished from selection as a consequence of behavior (Biro and Post 2008; Enberg et al. 2012; Biro and Sampson 2015). Experimentally fished crayfish (*Cherax destructor*) showed that trapping selectively captured fast growing juvenile crayfish, and that fast growth was strongly correlated with boldness (Biro and Sampson 2015). This finding is supported by studies showing that bold behavior is generally supported through artificial selection towards high growth rates (Johnsson and Abrahams 1991; Johnsson et al. 1996; Huntingford 2004; Sundström et al. 2004). However, the correlation between growth and behavior is not necessarily straightforward, because a range of studies have not found a robust relationship between boldness and production (Heg et al. 2011; Riebli et al. 2011; Nyqvist et al. 2012). Also largemouth bass selected over three generations for high vulnerability to angling showed lower growth rates under food restricted conditions compared to their low vulnerable counterparts (Redpath et al. 2009; 2010). However, Redpath et al. (2010) could show that high vulnerable largemouth bass have a higher metabolic rate, so that higher energetic costs might have caused reduced growth rates of these fish. The foraging ecology between the two selected lines also differed significantly. Low vulnerable largemouth bass captured more prey, attempted more prey captures and converted prey more efficiently into growth but also showed lower capture success, higher prey rejection rates and a shorter reactive distance (Nannini et al. 2011). Because aggression and vulnerability to angling is correlated in the selected lines of largemouth bass (Philipp et al. 2015), realized growth rates of fish might additionally depend on their foraging strategy, also explaining comparable correlations between growth and aggression in cichlids (Riebli et al. 2011). This together indicates a multiple trait-interplay as a mechanistic basis of vulnerability and that the relevance of life-history traits for vulnerability to angling should be seen in the context of underlying physiological and behavioral mechanisms and traits that correlate with life-history traits.

1.5 Abiotic effects

The mechanistic basis of vulnerability to passive fishing gears is not only a function of correlated phenotypic traits like morphology, behavior, physiology and life-history, but is also strongly influenced by environmental parameters and cues (Stoner 2004). Individual changes in activity, feeding and energy acquisition intensity are effected by temperature (Bigelow et al. 1999; Stoner 2004; Stoner et al. 2006; Damalas et al. 2007; Flores et al. 2008; Ortega-Garcia et al. 2008), light level, current velocity, ambient prey density and the presence of bait competitors (Stoner 2004), which might directly translate to catch rates. Depending on the preferred temperature, poikilothermic animals like fish change their activity including foraging activities with changes in temperature (Biro et al. 2010). Thus, it is possible that changes of environmental parameters can impact rapid changes in catch rates. Further, many species exhibit activity peaks at certain times of the day and also show seasonal patterns in movement and distribution (Hubert and O’Shea 1992; Grant et al. 2004; Creque et al. 2006; Kobler et al. 2008), all potentially influencing catchability of the fish. This can be the case during the spawning season, but might also be influenced by weather fronts, water depths, water-level fluctuations, turbidity and thermocline location (Berst 1961; Craig and Fletcher 1982; Hubert and Sandheinrich 1983; Craig et al. 1986; Pope and Willis 1996). Other abiotic parameters like wind speed, barometric air pressure and day-length are known to directly impact catch rates in angling fisheries (Bigelow et al. 1999; Margenau et al. 2003; Stoner 2004; Wall et al.

2009). Based on interactions of temperature, spawning success and foraging opportunities, latitude of the fished water body can influence capture probabilities as it was shown for piscivorous largemouth bass (Sutter et al. 2014). Similarly, catchability of walleye and pike was found to differ tenfold between northern and southern populations as a consequence of reduced foraging opportunities due to shorter growing seasons and the subsequent higher hunger levels of the fish in northern areas (Mogensen et al. 2014). Besides seasonal, geographical and weather impacts on fish and fisheries, the phase of the moon is known to influence the behavior of aquatic organisms (Hanson et al. 2008) which can be directly translated to catch rates of anglers within marine as well as freshwater environments (Lowry et al. 2007; Ortega-Garcia et al. 2008; Vinson and Angradi 2014). However, proximate analyses of abiotic impacts on angling catch rates, in particular within freshwater environments, are still rare.

Another environmental impact on catch rate and individual vulnerability of fish can be the fishing activities themselves (Klefoth et al. 2011). Angling-induced predation pressures through boat noise can impact fish activity and distribution (Jacobsen et al. 2014), because an approaching boat can be perceived by the fish similar to an approaching predator (Lima and Dill 1990). Thus, foraging activities can be impacted by both behavioral and physiological disturbance (Graham and Cooke 2008; Purser and Radford 2011; Bracciali et al. 2012), potentially influencing feeding times and energetic needs (Cooke et al. 2003; Brown et al. 2005), which in turn could directly impact vulnerability of the fish even in the absence of harvest (van Poorten and Post 2005).

A summary of factors known to influence vulnerability of fish in angling fisheries are summarized in Fig. 1.

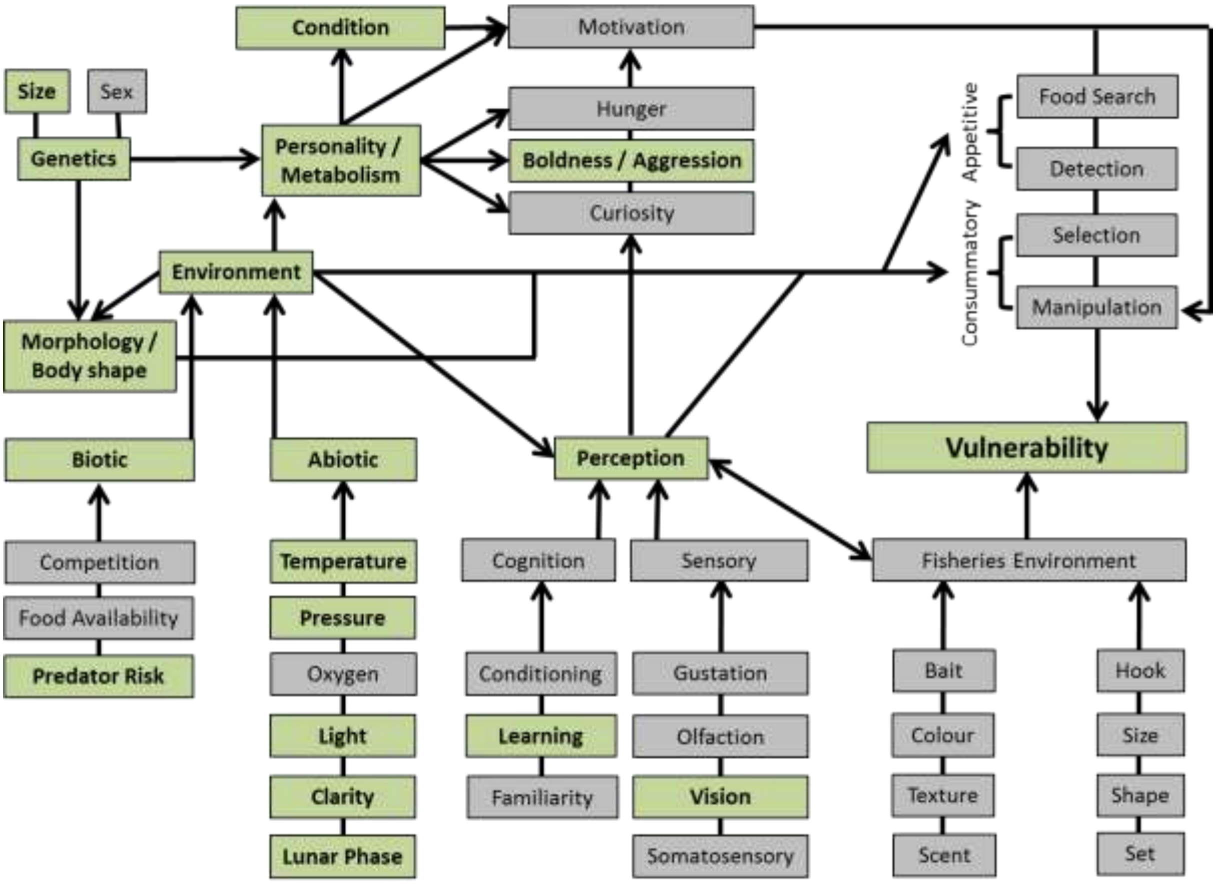

Fig. 1: Factors influencing vulnerability of fish in angling fisheries. Green boxes indicate parameters that were part of this thesis (adopted and revised from Lennox et al., unpublished). Consummatory and appetitive behavior of fish, and thus their vulnerability to angling gear is affected by internal and external factors that contribute to the probability of capture. The motivation to eat and to ingest a baited hook depends in an individual`s curiosity, hunger, and its individual level of boldness or aggression. These factors are again linked to the personality of the fish which is related to physiological parameters like metabolism and condition, but also to morphological parameters like size and body shape, genetics, biotic and abiotic environmental parameters like predation-risk, perception and learning, and the fisheries environment in interactions with gear technologies.

2. Fisheries induced selection vs natural selection

The selection differential S and selection gradients (β) are the central measures of selection in quantitative genetics (Hereford et al. 2004). They can be converted into mean standardized selection gradients (β_μ) to create comparable selection gradients for different traits and units, which is preferred for representing and interpreting selection in wildlife (Matsumura et al. 2012). The direction and strength of natural selection is usually driven by a growth-mortality trade-off where fast growers show high foraging and energy acquisition behaviors to grow more, but these behaviors also increase predation-risk (Enberg et al. 2012). Behaviorally mediated trade-offs between growth and predation mortality are likely to shape the evolution of intrinsic growth rate because all fish are usually affected by predation-risk, and susceptibility to predation increases with activity levels and usage of productive habitats (Biro et al. 2006). As a consequence, variability of life-histories within populations exists and the mean phenotype of the population will not reach maximal growth rates, although population fitness and resilience are maximized (Mangel and Stamps 2001). It is possible that a wide between-individual variability of growth rate produces similar life-time fitness (Mangel and Stamps 2001) and that this variability maintains adaptive evolution of growth under changing environmental conditions including fluctuating fishing pressure between years or decades.

Selection pressures induced by passive fisheries like angling and gill-netting will usually leave behind more timid and slow growing individuals (Edeline et al. 2007). This is true for both juvenile and adult fish, because high boldness and a fast life-style will continuously and positively influence vulnerability of these fish (Cooke et al. 2007; Härkönen et al. 2014; 2016; Alós et al. 2015b). Similarly, natural predation selects against maximal growth rates by removing individuals that display increased foraging efforts, thereby reducing survival fitness (Biro et al. 2006; Stamps 2007). As a consequence, natural and artificial (fishing) selection should often point into the same direction. In adult fish, however, additional fitness components like fecundity must be considered as fecundity can override survival as superior fitness component (Edeline et al. 2007). This is because larger fish have higher reproductive success and natural selection favors these fish, whereas artificial selection remains to select the large, fast growing and bold individuals (Alós et al. 2015a; b). Under these circumstances of concurring selection pressures acting on adult fish, different adaptive responses of life-history traits seem to be possible. Due to correlations among life-history and behaviors (Wolf et al. 2007; Realé et al. 2010), any changes in life-history to safeguard fitness in the face of exploitation may also lead to behavioral changes under size-selective harvest where individuals from highly exploited stocks can become more timid and less explorative than their conspecifics from unexploited stocks (Walsh et al. 2006; Uusi-Heikkilä et al. 2015).

Considering long-term data of adult pike under fluctuating natural- and harvest-induced selection through gill-net fisheries in Lake Windermere, Edeline et al. (2007) observed that trait changes moved in the direction imposed by the dominating selective force. A rapid shift towards fast growth of adult pike was observed after fishing pressure declined, and at the same time, size-and-age at maturation increased supporting the suggestion of a genetic trade-off between growth and reproduction (Law 2000). These interplays between natural selection and harvest selection can lead to fluctuating selection responses. In Windermere pike, natural selection was often stabilizing and fisheries selection using size-selective gill-nets was often disruptive because the smallest and largest fish were not vulnerable to the gear in use (Carlson et al. 2007; Edeline et al. 2007). In the context of an angling fishery, however, usually the largest individuals of a population are harvested so that directional selection against large body size is concurring with directional or stabilizing natural selection towards large body size.

Landi et al. (2015) used a size-structured life-history model to study the selective pressures exerted on commercially exploited fish stocks by different levels of fishing mortality and by different levels of selectivity for size and maturity. They found that harvested stocks under high exploitation rates of particularly large fish may split into two life-history types where one type takes advantage of early maturation and hence, shows lower than average growth rates whereas the opposite is true for the second type. This way, fisheries-induced disruptive selection might occur. A prerequisite for selection to turn disruptive is a large impact of growth, fecundity, and mortality trade-offs, and this happens more readily when the probability of early maturation becomes high (Landi et al. 2015). Because strong directional selection against large individuals enhances the probability of early maturation in exploited fish populations (Law 2000; Heino and Godø 2002), disruptive selection might be more likely to occur when natural selection and fishing-induced selection strongly act in opposite directions in a given trait (Edeline et al. 2009). Under these circumstances, genetic variability of the population increases together with an increasing variance of fitness related traits (Edeline et al. 2009). Thus, under relaxed fishing pressure, growth and energy acquisition behaviors should be mediated by the growth-mortality trade-off in both juvenile and adult fish and after maturation large fish have a fitness advantage due to higher fecundity and a relaxation of natural mortality. But when fishing pressure on adult and large individual fish increases, there is a chance of disruptive selection as a consequence of concurring and simultaneous strong selection pressures in opposite directions. As a result, bimodal distributions of selected and correlated traits like boldness and growth are conceivable (Fig. 2). For example, under high fishing pressure (i.e. a situation where many but not all large fish are harvested), intermediate phenotypes of boldness might perform poorly because they would not reach very high levels of reproductive fitness as a consequence of slower growth compared to extremely bold conspecifics, but they would still experience higher mortality than their very shy counterparts with slow growth and early maturation. Thus, extreme phenotypes might have the highest reproductive fitness (Kingsolver and Pfennig 2007). Further, once natural predation-risk decreases with increasing size, plastic responses towards the fitness landscape seem to be possible so that shy fish might become more proactive as a response towards higher fitness expectations under circumstances of high food availability (Edeline et al. 2007; 2009). Because fishing pressure adapts to the fishing opportunities and catch expectations (Arlinghaus 2006), this plastic response might be depressed again with increasing fishing pressure as an increasing number of individuals from the shy end of the behavioral spectrum are additionally captured in this scenario. If angling-induced selection either acts directional, stabilizing or disruptive, will finally depend on the interplay and strength of natural and artificial selection pressures within the given environment and fishery.

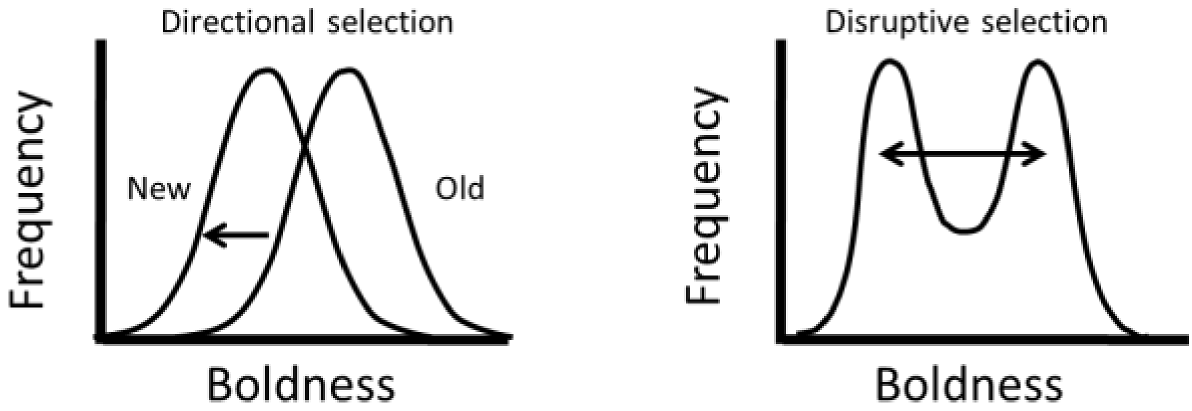

Fig. 2: Conceivable scenarios of angling-induced directional selection towards more timid juveniles and adults (left) and alternatively, non-linear selection of boldness-related traits as a consequence of concurring and simultaneous strong selection pressures (natural selection vs. angling-induced selection) in opposite directions at the adult stage.

3. Methodological issues

Many behavioral studies on fish and particularly studies determining personality aspects of fish have been conducted under laboratory conditions in aquaria (Mittelbach et al. 2014; Toms and Echevarria 2014; Näslund et al. 2015) whereas studies in the wild are comparably rare (e.g. Fraser et al. 2001; Biro et al. 2007; Adriaenssens and Johnsson 2013; Nakayama et al. 2016). Experimental setups used to measure personality traits can vary greatly, leading to unspecific interpretations of study results (Toms and Echevarria 2014; Näslund 2015). Fully controlled environmental conditions allow separation of intrinsic behavioral differences between individuals from plastic responses towards changing environmental conditions, but there is increasing evidence that behavioral observations in the laboratory often do not translate into behaviors of the same individuals under more natural conditions (Toms et al. 2010; Niemelä and Dingemanse 2014). Repeatability and consistency of behavior is strongly context dependent (Toms et al. 2010) and genotype by environment interactions can significantly impact behavioral expressions (Toms et al. 2010; Niemelä and Dingemanse 2014). In particular studies without explicit predation-risk stimuli more likely fail to identify consistent behavioral expressions in different environments (Fraser et al. 2001; Mittelbach et al. 2014; Cull et al. 2015) and between individual variation is not translated from the wild into the novel laboratory environment or vice versa (Niemelä and Dingemanse 2014). Because risk-stimuli can shape behavioral expressions (Brown et al. 2014; Elvidge et al. 2016), measurements of personality in fish are sometimes less repeatable if observations were exclusively conducted in the laboratory and studies sometimes fail to link the personality of fish with fitness-related consequences (Höjesjö et al. 2011). However, the consistency of behavioral expressions can be seen as a prerequisite for measuring direct selection on behavior (Allendorf and Hard 2009; Olsen et al. 2012) and it is known that behavioral traits predictive for the vulnerability of fish are repeatable over time (Bell et al. 2009; Chervet et al. 2011). In a fisheries context, all studies that failed to link boldness related behaviors to angling vulnerability were conducted in laboratory environments (Wilson et al. 2011; Kekäläinen et al. 2014; Vainikka et al. 2016) and angling vulnerability was measured in small laboratory tanks where all personality types could easily access the offered bait (Wilson et al. 2011). Therefore, behavioral measurements exclusively taken within controlled laboratory environments should be interpreted with caution, as long as no field validation of repeatable behavioral expressions exist (Niemelä and Dingemanse 2014).

Personality assessments within semi-natural conditions like ponds or large tanks, and particularly in the wild are problematic, because visual observations of the fish are often not possible. Indirect encounter mapping using passive integrated transponder systems (PIT) is one technological solution that can be used to overcome these problems (Krause et al. 2013). The tags are inexpensive and small, their lifetime is theoretically unlimited and human disturbance during behavioral observations can be precluded. However, problems can arise because the fish need to come very close to the vicinity of the reading antennae and multiple animals close to the same antenna reduce the efficacy of the tag reading as only one tag can be registered at the same time (Krause et al 2013). Irrespective of these constraints, PIT antennae systems have been widely used to observe fish behavior within natural environments (Lucas et al. 1999; Zydlewski et al. 2001; Riley et al. 2003; Skov et al. 2013) and under laboratory conditions (Brännäs & Alanärä 1993; Brännäs et al. 1994; Castro-Santos et al. 1996; Armstrong et al. 2001), including analyses of individual fish behavior in tanks to predict their vulnerability to angling gear (Härkönen et al. 2014; 2016; Kekäläinen et al. 2014). Comparable to standardized visual behavioral assessments within the laboratory, behavioral observations using PIT systems need to be conducted and interpreted carefully, because the link from presence-absence data generated by the PIT system to fish personality traits can be misleading. Therefore, calibration of the systems functionality and the effective measurement of the target traits are essential in such studies.

Methodological ambiguities also exist with respect to non-lethal assessment techniques of body composition in fish that indicate the physiological status and might therefore also predict the vulnerability towards baited hooks. A range of non-lethal assessment methods of proximate body composition, usually measured as the relative amount of moisture, lipid, protein, and ash within fish flesh have been developed in the past. Length-weight-regression-based condition indices such as Fulton`s condition factor (Ricker 1975), relative condition factor (Kn) (Le Cren 1951), and the ratio of dry mass to wet mass of an individual (Hartman and Brandt 1995) are common examples. The latest technical developments for estimating proximate body composition and/or energetic status of fish were based on the inverse correlation of lipid content and water content (Caulton and Bursell 1977; Schreckenbach et al. 2001). In these applications water content in fish flesh is measured using electric currents [bioelectric impedance analysis, BIA, Cox and Hartman (2005)] or microwaves in handheld devices [fat meters, FM, Crossin and Hinch (2005)]. However, all of these assessment methods have been criticized in the past (Cone 1989; Kent 1990; Hendry et al. 2001; Pothoven et al. 2008; Hanson et al. 2010) so that non-lethal assessment methods of proximate body composition need to be carefully calibrated in the specific content of usage to become a reliable tool for studies on angling-induced selection.

4. Objectives and thesis structure

The main objective of my doctoral thesis was to disentangle the mechanisms behind fish vulnerability to angling gear and to predict the ecological and evolutionary consequences of angling-induced selection. Specifically, my objectives were i) to develop a theoretical framework for fishing-induced evolution of behavioral and underlying physiological traits, ii) to develop material and methods useful for measuring phenotypic traits that influence fish vulnerability to angling gear including laboratory and field approaches, iii) to estimate the selection gradients imposed by angling in several behavioral, physiological, morphological and life-history traits, and iiii) to study the fitness-consequences of trait-selective and angling-induced harvest in wild populations to predict. For this I used four fish species with different life-history strategies as a model. Namely these species were common carp (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), amago salmon (*Oncorhynchus masou ishikawae*) and pike (*Esox lucius*). The structure of my thesis is based on five components to obtain the research goals (Fig. 3). As a first step, 1) the mechanistic pathways of fishing-induced evolution by selection on angling vulnerability are described, second 2) the methodological reliabilities of different phenotypic description techniques are evaluated, followed by 3) descriptions of the individual`s phenotype based on behavior, morphology, physiology and life-history and their link to individual vulnerability to angling, 4) experiments to further our understanding of the mechanistic basis of vulnerability to angling and to calculate selection gradients imposed by angling, and finally 5) I tested for the natural fitness of these individuals based on both survival and reproductive fitness. Most of the findings are published or corresponding manuscripts are submitted. However, some preliminary results, namely the metabolic basis of vulnerability to angling in carp and the survival probability of vulnerable and invulnerable carp towards natural predators are presented in the following chapters without being published or submitted yet.

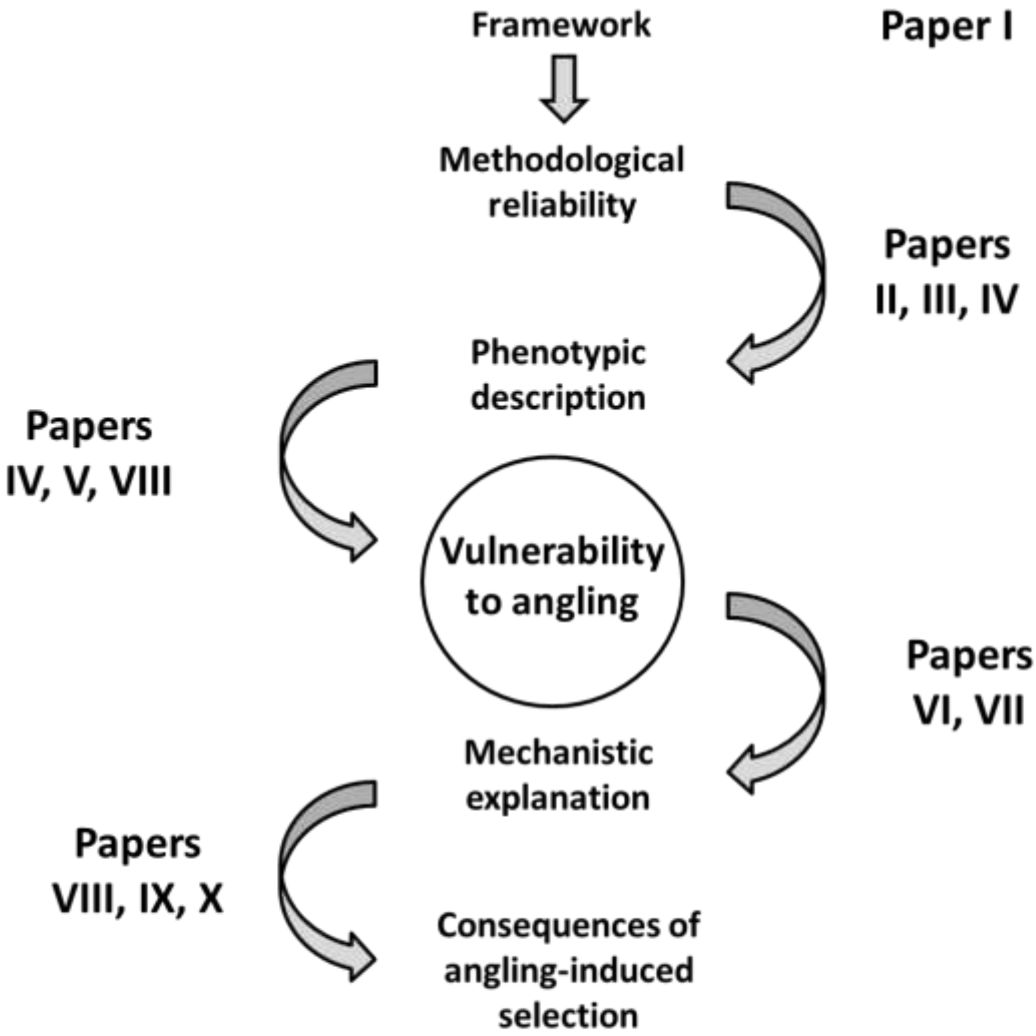

Fig. 3: Structure of my thesis aiming to disentangle the mechanisms behind fish vulnerability to angling gear and their ecological and evolutionary consequences with corresponding papers.

In detail, the thesis addressed the following research questions:

Note: Bold roman numbers identify the relevant paper(s) from the list of papers where several manuscripts may be relevant to answer single research questions.

Framework (**paper I**)

Methodological issues

- How reliable are non-lethal assessment methods of energetic state and behavioral measurements of exploration and boldness under laboratory conditions **(II, III, IV)**?

Phenotypic description and angling induced selection

- How do individual behavior, growth, morphology and energetic state influence vulnerability to angling and is behavior correlated with growth **(IV, V, VIII)**?
- How does metabolic scope influence vulnerability to angling (unpublished)?
- How strong is angling-induced selection acting on different phenotypic traits and in which direction does selection act on **(IV, V, VIII)**?

The mechanistic basis of vulnerability to angling

- How important is energy-acquisition related behavior for determining vulnerability to angling gear **(I, VI)**?
- How does learning influence catch rates **(VI)**?
- How do environmental factors influence catch rates **(VII)**?

Consequences of angling-induced selection

- Is survival fitness of juvenile fish correlated with vulnerability to angling (unpublished)?
- Is reproductive fitness correlated with vulnerability to angling **(VIII)**?
- What are the potential outcomes of angling-induced selection at the population level **(IX, X)**?

5. Framework

There is increasing knowledge that besides an evolution of life-history traits, fishing-induced evolution of behavioral and underlying physiological traits might occur as a consequence of selection acting directly on behavioral, rather than on life-history traits in passive fishing tactics like angling (see introduction and **paper I**). Regardless of the important role of behavior in determining vulnerability to fishing gear (see introduction), the behavioral dimension of fisheries-induced evolution just recently attracted notice to the scientific community (**paper I**). The mechanistic pathway of fishing-induced evolution by selection on fishing vulnerability is described in **paper I** and summarized in Fig. 4. This framework was used as a theoretical basis to experimentally test for the strength and direction acting on behavioral, physiological, morphological, and life-history traits in passive angling fisheries and to predict its ecological and evolutionary consequences.

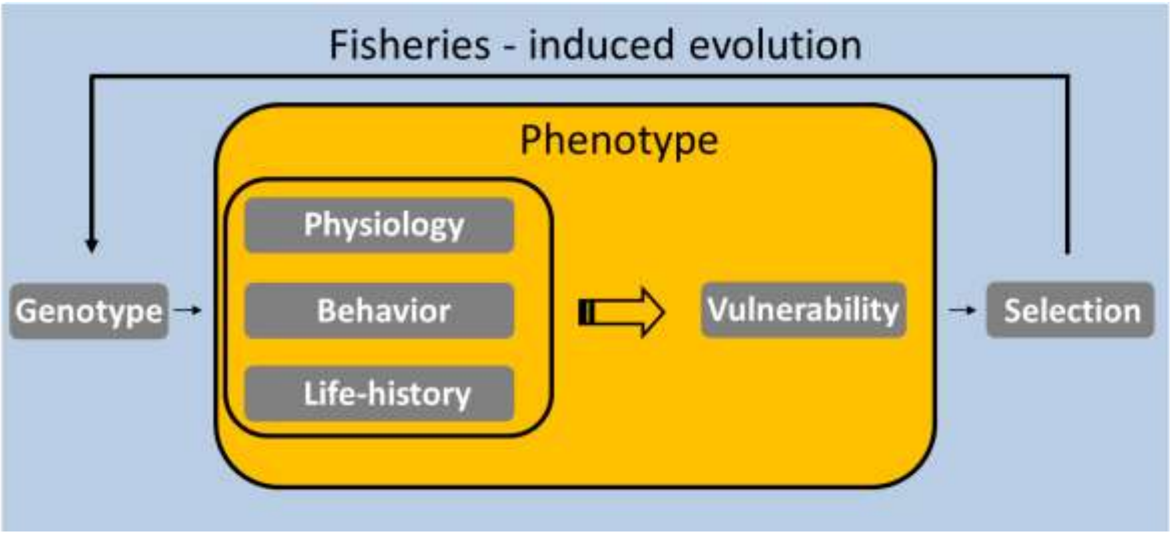

Fig. 4: Vulnerability to capture is considered a heritable trait as part of the fish`s phenotype. Vulnerability to capture comprises a bundle of physiological, behavioral, and life-history traits that jointly determine vulnerability to capture. In passive fisheries like angling, vulnerability to capture is largely determined by specific behavioral patterns rather than by body size-related life-history traits per se. Due to genetic correlations between behavioral, physiological and life-history traits, fisheries-induced selection on behavioral traits might alter physiologies and life-histories, but behavior might also change in response to selection on correlated life-history or other traits (**from paper I**).

6. Methods overview

6.1 Model systems

Common carp was the most chosen model species in my experiments. This species was used because it represents a common and world-wide existing omnivorous species with high relevance in European and German angling fisheries (Arlinghaus and Mehner 2003; Arlinghaus 2007), and because carp are known to be very robust against negative stimuli (Reilly et al. 2008) like repeated measurements and capture events. This robustness was required in experiments for **papers II, III, IV, and V** and in additional experiments analyzing metabolic scope and individual survival probability towards natural predators. During all studies, scaled and mirror carp were used. Scaled carp are fully scaled, representing the original morphotype of wild common carp, whereas mirror carp have much less scales, representing a morphotype that is strongly domesticated and highly adapted to low-risk pond conditions (Balon 2004; Matsuzaki et al. 2009). By choosing these two genotypes of carp, inter-individual variability of phenotypic expressions in this species was maximized. All carp used during my studies were raised at a commercial fish hatchery (Fischzucht Wegert, Ostercappeln, Germany) in the same common-garden pond environment. Parental fish descended from two selection lines (one with scaled morphotypes and one where scaled morphotypes were previously crossed with highly domesticated mirror carp). Young-of-the-year mirror carp could only develop as a result of two breeders from the strain originally crossed with domesticated mirror carp (Kirpichnikov and Billard 1999) **(papers II, III, IV, and V)**. At an age of 10 months, the pond was drained and juvenile carp were transported to the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin for further experimentation. Holding and feeding of the fish was similar in all papers and during all time making results of the different studies comparable.

In **paper V** we used amago salmon to investigate the long-term consequences of angling-induced selection on body size, growth, and behavior. This species was chosen because it is an endemic and non-migrating salmonid species in central Japan where individuals from two separated river-stretches with continuously and strongly differing exploitation rates over more than 6 decades were available for experiments. These circumstances made amago salmon a suitable model system to investigate phenotypic determinants and long-term consequences of angling-induced selection in a salmonid species.

In **paper VII** we used pike to identify abiotic and fishing-related correlates of angling catch rates. Piscivorous pike were chosen due to their paramount importance in recreational fishing within the northern hemisphere (Beardmore et al. 2015) and because catch-rates in pike are comparably high (Dorow et al. 2011), providing a suitable model system for analyses of catch-rates in relation to environmental conditions.

Finally in **paper VIII**, two lines of piscivorous largemouth bass, selectively bred over three generations for either high or low vulnerability to angling were used to determine correlations between vulnerability to angling and behavior, intensity of parental care, and reproductive fitness. These selected lines of largemouth bass are known to differ in their physiology (Cooke et al. 2007; Redpath et al. 2010), growth (Redpath et al. 2009), and food consumption rates (Cooke et al. 2007, Nannini et al. 2011), but not in their locomotory activity (Binder et al. 2012) where the heritability of the combined trait "vulnerability to angling" has be quantified at $h^2 = 0.146$ (Philipp et al. 2009). Therefore, these selected lines of largemouth bass can be seen as a perfect model system, particularly for studying fitness consequences of angling-induced selection.

6.2 Phenotypic trait assessments

As a first step, phenotypic trait variation in carp **(papers II, IV, VI)**, amago salmon **(paper V)**, and largemouth bass **(paper VIII)**, both within the laboratory and under semi-natural conditions in ponds was assessed. The main focus was set on behavioral measurements like boldness and aggression along with morphological measurements like body shape, life-history measurements like growth, and physiological measurements like energetic state and metabolic scope. These analyses were then used to calculate selection gradients **(paper IV)**, to compare the outcome of long-term angling-induced selection **(Paper V)** or to test my fitness objectives **(Paper VIII and unpublished data)**, collectively explaining the mechanistic basis of vulnerability to angling gear and the potential ecological and evolutionary consequences of trait-selective harvest.

6.3 Carp

6.3.1 Morphology

I examined the body shape and the shape of the head of each individual carp as a potential determinant of angling vulnerability using a landmark-based approach following the methods outlined in Rohlf and Marcus (1993) in **paper IV**. A total of 16 landmarks along the left body-side of each individual fish were digitized from standardized pictures and corrected for arching effects (Valentin et al. 2008) before principal component analyses of procrustes shape co-ordinates were used to analyze the shape of the fish. Residuals of linear regressions between PCA scores and total length of the fish were used as potential indicator of individual vulnerability to angling gear. In addition, TL of the fish was considered as important phenotypic expression, potentially influencing vulnerability in **paper IV**, and survival fitness in additional experiments aiming to predict individual fitness of the fish in the vicinity of a natural predator (unpublished).

6.3.2 Behavior

Classic behavioral measures of individual juvenile carp personality as defined by Réale et al. (2010) were conducted using variants of an open field test (Budaev 1997) under fully standardized laboratory conditions in **paper IV**. The aims of these experiments were to define individual behavioral expressions of the fish that might also explain the individual vulnerability to angling gear. Carp were investigated for their exploration behavior, boldness, feeding activity after disturbance, and sociability within four different setups in aquaria that were installed in a climate chamber at 20° C to control for effects of temperature on the expression of personality (Biro et al. 2010). These trials were repeated after exactly six weeks to determine repeatability and consistency of behavioral expressions in a standardized environment before tests for angling vulnerability were conducted in ponds using the same individual fish.

To study the behavior of carp within large laboratory tanks (10 m x 4 m x 1 m; *L x W x H*) and within comparably sized semi-natural ponds (12 m x 5 m x 1 m; *L x W x H*), I used an automatic passive integrated transponder (PIT) system consisting of a shelter structure and two feeding spots in different distances to the shelter (Fig. 5). Boldness-related behaviors were examined based on the time spent sheltering and the number of visits at the two feeding spots where the distant feeding spot was assumed to be perceived the most dangerous by the fish as carp had to cross a large open area before entering it, similar to an open-field test (Budaev 1997). This PIT system was used in **papers II and IV**. **Paper II** tested for the importance of genotype x environment interactions and the presence of predation-risk stimuli on boldness assessments of fish by comparing the number of visits at the two feeding spots and the time spent sheltering between scaled- and mirror carp within the large laboratory tanks and the ponds. Because scaled and mirror carp are known to differ in their boldness expressions due to differences in their domestication history (Matsuzaki et al. 2009), **paper II** served as a control for behavioral determinates of angling vulnerability in **paper IV** that were later used to calculate selection gradients. Functionality of the system was tested through comparisons of visual observations of focal fish, equipped with external floy tags and internal PIT tags, and corresponding data provided by the PIT system as described in **paper IV**.

The swimming activity (# of side changes), feeding behavior (# of items eaten, time to ingest the bait) and food preferences (pellets vs. corn) of scaled and mirror carp as possible mechanistic explanations for differences in angling vulnerability between generally bold (mirror carp) and comparably timid genotypes (scaled carp) were compared in a two-way-choice experiment (Holbrook and Schmitt 1988) where individual fish were investigated in aquaria in **paper VI**. Using six replications, two different food resources (pellets and corn) were placed in each side of the aquaria and the swimming activity, time until feeding, food ingestion rate and food preferences were measured, aiming to understand the mechanistic basis of vulnerability to angling based on behaviors related to energy-acquisition.

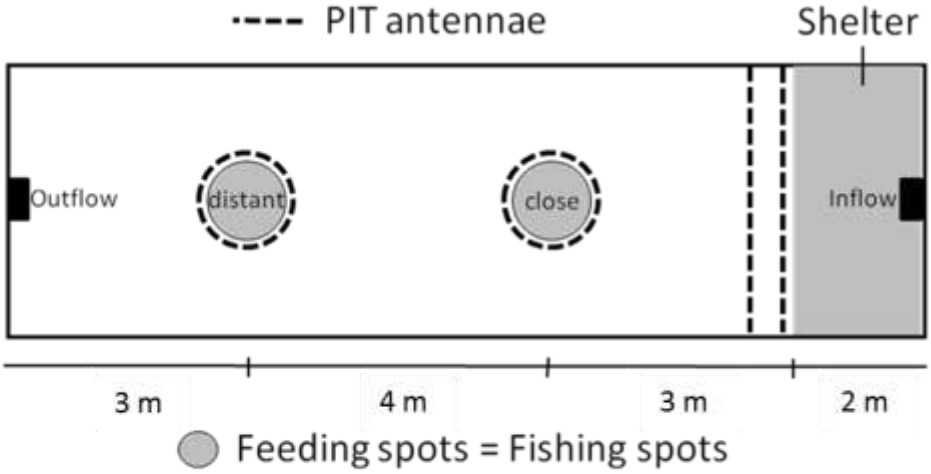

Fig. 5: Experimental setup for behavioral observations under semi-natural pond conditions and within similar-sized laboratory tanks in **papers II and IV**. Within this setup, boldness was defined by low sheltering times and high number of visits at the close and the distant feeding spot (circles).

6.3.3 Physiology

To non-lethally assess the body composition of carp I used relative condition factor (K_n), bioelectric impedance analyses (BIA) and fat meter (FM) measurements. In **paper III** I compared the performance of these three methods. Because water and lipid contents in fish flesh are inversely correlated and a higher dry mass is positively correlated with energetic density and hence condition (Caulton and Bursell 1977), the calibration procedure was used to predict relative dry mass of the fish. Based on my comparative approach, fat meter measurements were further used in **paper IV** to investigate the energetic status of my experimental carp at the time of stocking as a potential indicator for their individual vulnerability to angling gear.

Subsequent to all experiments in **paper IV** a subsample of caught (N = 6, mean TL ± SD = 213 ± 1.2 mm) and uncaught (N = 6, mean TL ± SD = 231 ± 1.7 mm) carp representing scaled (N = 5, mean TL ± SD = 220 ± 1.0 mm) and mirror (N = 7, mean TL ± SD = 223 ± 2.1 mm) genotypes were tested for their metabolic scope using flow-respirometry (unpublished and described in detail below). The experimental setup was similar to Ohlberger et al. (2005) and consisted of a modified Brett-type tunnel respirometer with a measuring circuit (25 l) which contained a swimming chamber of 15 cm in diameter and a ventilation circuit with a volume of 125 l. The respirometer was build up in a climate chamber (inner dimensions: 276 cm x 276 cm x 210 cm; ILKAZELL Isoliertechnik GmbH, Zwickau, Germany) to control temperature ± SD at 18 ± 0.2 °C. Oxygen concentration and temperature were measured with a fixed TriOximatic 701 sensor (WTW, Weilheim, Germany) coupled to an oximeter (Oxi 171; WTW, Weilheim, Germany) that allowed automated flushing and measuring periods. Oxygen concentration, temperature and ventilation status were automatically recorded every 6 seconds.

Fish were held in round tanks (diameter of 1 m, water exchange every 3 days) with aerated water and were starved for 48 h before they were weight and transferred to the respirometer. Within the swimming chamber fish were allowed to acclimatize for a minimum of 1 day at a flow velocity of 0.5 body length (bl, similar to TL) s^{-1}. Subsequently flow velocities of 0.5, 0.75, 1.0 and 1.5 bl s^{-1} were run for approximately 24 h each. After removal of the fish a blank value was determined to consider bacterial ventilation which accounted for up to 36 % of total respiration and was therefore comparable to values observed by Ohlberger et al. (2005). After measuring periods of approximately 8 – 18 min (dependent on flow velocity) aerated fresh water was pumped into the measuring circuit when a lower threshold value of 86.5 % oxygen saturation was reached so that fish were never exposed to hypoxia.

According to Redpath et al. (2010) a linear regression between variations in oxygen partial pressures and time was calculated and the slope of the regression line (*k*) was then used to calculate oxygen consumption as: $M\text{o}_2 = kV_{\text{resp}} \text{ x } M^{-1}\alpha$ where $M\text{o}_2$ is the oxygen consumption in mg O_2 kg^{-1} h^{-1}, V_{resp} is the volume of the respirometry chamber in L, M is the fish mass in kg and α is the solubility of O_2 in water at a given temperature. Metabolic scope in mg O_2 kg^{-1} h^{-1} was then calculated as the difference between the minimum oxygen consumption rate at a flow velocity of 0.5 bl and the maximum oxygen consumption rate at a flow velocity at 1.5 bl observed overnight. Values generated at flow velocities of 0.75 bl and 1.0 bl and values generated during the day were not considered for further analyses as regular spontaneous activities of the fish occurred, likely as a consequence of humans entering the climate chamber during experimentation.

I compared the metabolic scope in mg O_2 kg^{-1} h^{-1} between caught and uncaught individuals and between scaled and mirror carp using ANOVA and used Pearson`s correlation to test for relationships between metabolic scope and boldness in ponds as determined by the number of visits at the distant feeding spot (see chapter 6.3).

All data were LN-transformed to reach normality and analyses were conducted using SPSS version 20 (IBM, Armonk, NY, USA).

6.3.4 Life-history

Life-history traits are among the prime targets of fisheries-induced selection (Heino et al. 2015) and I measured growth of juvenile fish to test for the hypothesis that growth rate is positively correlated with vulnerability to angling through a positive correlation with energy-acquisition related behaviors (Enberg et al. 2012; Biro and Sampson 2015). In **paper IV**, growth of juvenile carp was assessed after angling experiments in ponds were finalized (see below). The fish were fed commercial fish pellets at a ratio of 1 % of the initial biomass at the time of stocking per day. Therefore, growth of the fish was measured under food-restricted conditions.

6.3.5 Vulnerability to angling

I tested the individual vulnerability to angling of carp in papers **II, IV, and VI.** This was done either in large laboratory tanks **(paper II)** or in semi-natural ponds **(papers II, IV, and VI)**, aiming to test for boldness-related expressions of the fish in the vicinity of angling-induced predation-risk **(paper II)**, to identify the strength and direction of phenotypic selection through angling activities **(paper IV)**, or to assess the impacts of learning, foraging behavior and food preferences as potential mechanistic explanations for differences in individual vulnerability to angling gear **(paper VI)**. In any case, the angling procedure was standardized to the same protocol, making results of the different papers comparable. Corn was used as bait on a “bolt-rig”, usually used by specialized carp anglers, because this method ensures shallow hooking of the fish (Rapp et al. 2008). Fishing was alternated between the close and the distant feeding spot (compare Fig. 5) and fish were identified and released within less than 30 seconds. To compare the strength of selection caused by angling among different phenotypic traits in **paper IV**, mean standardized selection gradients ($\beta\mu$) based on logistic regression coefficients were estimated following Matsumura et al. (2012). The $\beta\mu$ is preferred for representing selection in the wild and it represents the relative change in fitness that results from doubling of the trait value (Matsumura et al. 2012). The measure allows comparisons of the strength of selection acting on several traits that differ in units, means and variance (Hereford et al. 2004; Matsumura et al. 2012) and was therefore used in my studies.

6.3.6 Survival fitness

After finalizing all experiments from **papers IV and VI**, a total of N = 136 carp with known vulnerability to angling (N = 39 were previously captured) were equally distributed among four ponds at IGB in November 2010 (unpublished). Two ponds only contained carp to test for predator-independent mortality whereas the other two ponds were additionally stocked with four large pike to test for survival fitness of previously caught and uncaught carp in the vicinity of a natural predator. Mean total length of the carp ± SD was 206 ± 18 mm (range 165 – 261 mm) and the length of the pike ranged between 750 mm and 990 mm. These large pike were caught by angling in the natural Vätersee approximately 100 km north of Berlin. Pike of this size can easily handle prey with maximal body depths above 80 mm (Nilsson and Brönmark 2000) and the mean body depth of stocked carp was 53.2 ± 4.7 mm (range 42 – 68 mm) so that the gape size of pike-predators was not a limiting factor and for each pike the possibility to ingest any of the experimental fish was guaranteed at any time. The experimental setup within the ponds principally followed the pond–setup in **paper IV** where a shelter structure at one end of the pond was established. Because no additional prey fish were stocked during this experiment, carp were protected from pike by a netting wire installed at the entry of the shelter structure thereby separating the save shelter from the rest of the pond where pike were allowed to freely move. Within the netting wire a total of eight little holes allowed carp to switch between the two habitats within the pond. Because no food was provided under shelter, carp were forced to take the risk of moving into the open area of the pond to forage on provided pellets (compare **paper IV**) whereas pike could only forage on risk-taking carp. After five months the ponds were drained and surviving carp and all pike were retrieved and identified by their individual PIT-tag that was implanted during previous experiments.

I compared survival fitness between previously caught and uncaught individuals using a generalized linear mixed model with predators nested within ponds as a random factor and vulnerability to angling (yes/no) and total length of the carp at the time of stocking as fixed factors. Analyses were conducted using library “lme4” (Bates et al. 2015; http://cran.r-project.org/web/packages/lme4) in the R package version 3.1.2. (R Development Core Team 2008).

Another subsample of N = 16 carp (9 previously captured and 7 previously uncaptured fish, mean TL ± SD 291 ± 19 mm and 266 ± 18 mm, respectively) with known vulnerability to angling from **papers IV and VI** were stocked into the natural Döllnsee (25 ha) (unpublished) which was previously equipped with an automatic CDMA telemetry system (Baktoft et al. 2015). Calibration of the system confirmed a high functionality (Baktoft et al. 2015) and in this case the system was solely used to classify stocked carp as dead or alive on a daily basis. All carp were anesthetized in a clove oil-ethanol solution (1:9; 0.4 ml/l) and I implanted ultrasonic transmitters (M-48; 2.7 g in air; 15 s burst rate; 412 d calculated life-time; Lotek Wireless Inc., Newmarket, Ontario, Canada) into the body cavity following the protocol described by Hühn et al. (2014). After transmitter implantation fish were allowed to recover in 80 l plastic tanks filled with aerated water until they were stocked into the littoral of the lake. A fish was classified alive when continuous swimming activity could be observed and carp were considered dead when no signal could be detected anymore. Days of survival (max. 400 d) were compared between previously caught and uncaught individuals using ANOVA. To reach visual normality, data were LN-transformed before analyses were conducted using SPSS version 20 (IBM, Armonk, NY, USA).

6.4 Amago salmon

6.4.1 Behavior, growth and vulnerability to angling

Predator display tests in a laboratory tank were conducted and replicated six times to examine boldness-related behaviors of wild-caught juvenile amago salmon selected from two separated river stretches, varying in their degree of angling-induced exploitation in **paper V**. Groups of fish were placed in a tank that was partly covered by a shelter and a cormorant model was displayed to the fish. After three seconds, all fish hiding under shelter were separated from the other fish so that the individual behavioral decision, either to seek refuge or to remain in the potentially dangerous open water could be measured. The mean hiding score of individuals from the high pressure and low pressure streams was then used to predict vulnerability to angling of these fish.

In **paper V**, individual growth of amago salmon was tested over a period of 36 days where fish were fed ad libitum. Therefore, this growth assessment tested for intrinsic growth rate without any food limitations. However, growth of the amago salmon was also tested under more stressful conditions over 24 days with repeated and explicit predation-risk stimuli induced to the fish via predator display tests. Food ratios were again ad libitum.

To compare the body size-dependent vulnerability to angling between fish in high- and low angling-pressure streams, experimental angling for amago salmon within the two river stretches was conducted in **paper V**. The angling equipment consisted of a 1.5 m long pole without a reel and frozen shrimp as bait. Juvenile amago salmon were additionally tested for their individual vulnerability after phenotypic description within the laboratory. Here, angling took place in a small tank (33 cm x 46 cm x 30 cm; *L x W x H*) ensuring fully controlled environmental conditions.

6.5 Largemouth bass

6.5.1 Behavior, vulnerability to angling and reproductive success

Male largemouth bass from two selection lines for either high- or low vulnerability were stocked in six replicated ponds and scored for their individual aggressive behavior and for their nest-guarding activities in **paper VIII**. Both, aggression and nest-guarding activities are known to be correlated with reproductive fitness in this species (Philipp et al. 1997; Suski and Philipp 2004) so that these behavioral measurements could later be used as additional indirect indicators of reproductive success. Aggression was tested by counting the number of strikes of each individual male fish on three different lures without hooks where the maximum number of strikes per replicated session was 15. Levels of parental care investment were assessed from shore, observing the individual nest-guarding males. Behavior of the fish was classified as “present on the nest and fanning eggs”, “engaged in chasing egg-predators” and “absent from the nest”, providing insights into the individual nest-guarding intensity. In addition, TL of the fish was considered as important phenotypic expression.

To examine the impacts of angling-induced selection on reproductive success in **paper VIII**, males from the F5 generation of two lines of largemouth bass that had previously been artificially selected to exhibit high or low vulnerability to angling lures over three generations (Philipp et al. 2009) were used in replicated pond experiments. Males from the two selection lines were stocked together with wild-caught females and from April - September 2009 the parental care behavior (e.g. aggression, nest-guarding intensity) and the reproductive success (e.g. number of eggs on the nest) of males were visually observed. In autumn the ponds were drained and based on a random subsample of offspring, relative reproductive success of the fish was examined using microsatellite markers.

6.6 Pike

6.6.1 Environmental correlates of catch rate

In **paper VII**, unstandardized angling for pike was conducted in the natural Döllnsee (25 ha) to identify abiotic and fishing-related correlates of catch-rates in angling fisheries (see Kobler et al. 2008 for a detailed description of the study lake and its fish population). A total of 25 anglers targeted pike, of which three anglers were fishing almost every day from end of May until mid-September, thereby covering all weather conditions from late spring to early autumn. Used lures, baits and fishing spots were not standardized to allow spontaneous adjustments of fishing techniques and to reflect “normal” conditions of angling activities. Catch-rates were then correlated to abiotic environmental conditions like water-temperature, wind-speed, wind-direction, rainfall, hours of sunshine, air-pressure, humidity, moon-phase, fishing effort during the last days, and time of the day.

7. Main findings and discussion

7.1 Reliability of phenotypic descriptions

I repeatedly used automatic PIT systems to observe sheltering activities and the number of visits at two feeding spots, i.e. foraging activity, in both large laboratory tanks and ponds (**paper II and IV**). In **paper IV** I could show that my automatic PIT systems provided reliable data for boldness-related expressions of the test fish. Although the PIT system consistently underestimated the number of visits at the feeding spots and the time spent sheltering, inter-individual relative habitat distributions among focal fish were reasonable reflected by the data as confirmed by high correlations between the real and the measured number of visits at the two feeding spots and the time spent sheltering. Hereby the numbers of conspecifics present at the specific habitat, i.e. feeding spots and shelter, significantly and negatively influenced detection probabilities so that PIT antennae systems are limited in the total number of fish that can be observed at the same time. Because all experiments using the PIT system (**papers II and IV**) were performed with similar numbers of fish within each replicated pond or tank (N = 40 individuals), my results are comparable between studies and potential limitations of the PIT detection probability were similar between studies.

Laboratory based quantifications of behavior assume that personality expressed in novel environments predicts personality expressed in the wild (Niemelä and Dingemanse 2014). However, there is increasing evidence that boldness assessments within a benign laboratory are less repeatable compared to studies under more natural conditions and with an explicit predation threat involved (Bell et al. 2009; Toms et al. 2010). My approach of testing for the reliability of behavioral measurements under laboratory and semi-natural conditions in ponds was therefore based on known differences in bold behavioral expressions between genetically different and highly domesticated mirror carp and less domesticated wild-like scaled carp in **paper II**. Because consistent and repeatable individual variation of behavior is a prerequisite when aiming to show a selection response, as I did it in **paper IV**, rapid assaying of behavior in the laboratory might be unsuitable as this can introduce serious bias in personality assessments and can lead to misinterpretation of its ecological and evolutionary consequences (Biro 2012; Niemelä and Dingemanse 2014). My findings in **paper II** confirmed that highly domesticated mirror carp are generally bolder compared to their less domesticated scaled conspecifics, particularly in relation to feeding behavior in the presence of natural or angling-induced predation-risk and less pronounced also in relation to shelter usage. However, I also confirmed that rapid behavioral assessments under laboratory conditions can lead to misinterpretation of results as I did not find clear differences in boldness between scaled and mirror carp when fish were observed under standardized conditions without predation-risk in the laboratory only, indicating strong genotype x environment interactions in boldness behavior of carp. When behavioral observations were conducted in ponds, behavioral differences between the two groups of fish were consistent and clear whereas in the laboratory, artificial predation-risk stimuli were necessary to obtain comparable results. Similarly, repeatability of boldness-related expressions of carp in aquaria in **paper IV** was very weak and not significant, indicating that despite obvious benefits of controlled behavioral phenotyping in the laboratory, field experiments are of paramount importance if ecological and evolutionary consequences of behavioral expressions are to be studied. I showed that simple applications of a variant of a classical open-field test, which is often assumed to reliably measure boldness in fish (e.g. Budaev 1997; Brown et al. 2007) in aquaria or large laboratory tanks can provide inconclusive or even misleading results in terms of adopted boldness differences among fish with known differences in behavioral expressions as a consequence of genotype x environment interactions **(paper II)**. Randomness of behavioral expression tends to be predominately pronounced in non-threatening situations (Alados et al. 1996) and therefore, studies focusing on selection responses of behavior in fish should be conducted within the original evolutionary environment (Kawecki and Ebert 2004; Walling et al. 2004) or in ecological conditions reasonably close to those of the original environment as I did it in **papers IV, VI and VIII**. If this is not possible, repeated measurements of boldness should include observations under predation-risk (Toms et al. 2010), as taken into account in **paper V**.

Hunger and individual physiological status like body fat content can influence the behavior of fish (Stoner 2004) and therefore potentially also their individual vulnerability to angling-like gear (Løkkeborg et al. 2010). In **paper III** I tested for the reliability of three non-lethal assessment methods of body composition and energetic status of carp to be able to add these physiological characteristics as potential predictors for angling vulnerability to my other studies. My experiment showed that fat meter (FM) consistently performed better than bioelectric impedance analyses (BIA) and the relative condition factor (K_n) to predict relative dry-mass in whole body carp and dorsal white muscle of carp (FM measurements explained up to 81.4 % of total variance). Due to a very strong correlation of dry mass and lipid content, and particularly energy density, the FM approach was found to be useful for non-lethal assessments of the energetic status of carp. Thus, in comparison to other methods, FM provided the most robust and repeatable results which has been confirmed by other studies before (Pothoven et al. 2008; Hanson et al. 2010), and therefore I used this method in **paper IV** to non-lethally predict the energetic status of carp as potential trait predicting vulnerability to angling.

7.2 Phenotypic correlates of individual vulnerability to angling in benthivorous fish

In **paper IV** I used benthivorous carp as a model species to disentangle the relative strength, direction and importance of angling-induced selection against behavioral, physiological, morphological and life-history traits and I found boldness in ponds to be the most important trait under selection in angling fisheries as indicated by 1.5 times greater standardized selection gradients acting on boldness ($ß_{\mu}$ = -0.655) compared to juvenile growth ($ß_{\mu}$ = -0.424). Whereas boldness observed under laboratory conditions was unrepeatable and energetic status and body shape were not or only weakly related to angling vulnerability, a significant correlation between boldness in ponds and growth (Pearson`s r = 0.31, P = 0.002) supported the hypothesis that direct selection on boldness will also induce selection on growth rate in benthivorous carp as it has also been shown for crayfish (Biro and Sampson 2015), and as it has been previously argued by Biro and Post (2008). However, independent evolution of these traits is also possible and needs further investigation. My negative findings for the energetic status of the fish as a predictor for angling vulnerability are probably based on the short-term nature of this parameter. It is known that hunger influences catchability of fish (Raat 1991; Herrman et al. 2013), and likely the energetic state of the focal fish quickly changed after release into my experimental ponds so that measurements of relative body fat content at the time of stocking did not properly reflect the energetic status of the fish at the time of capture. Morphological parameters only added little to the suite of phenotypes under selection in my experimental fishery, likely because parameters like body shape and head size simply act as a surrogate for behavior (Alós et al. 2014). However, comparable to the findings of Alós et al. (2008) and Alós et al. (2014), I found weak evidence that larger heads and mouths positively influenced vulnerability of the focal carp independently of the fish body size.

These results are novel because **paper IV** revealed the relative importance of behavioral selection in comparison to morphological and life-history traits in angling fisheries. However, strong selection on risk-taking and energy-acquisition related behaviors, i.e. visits at a feeding spot with the greatest distance to a save shelter, only emerged when angling was conducted for a longer period of 20 days. By contrast, when fishing was conducted for only seven days, the strongest selection pressure acted on total length, albeit size of the focal fish only varied little. It can thus be assumed that under more natural conditions with fish from different size-classes being exploited at the same time, size selectivity is present in passive angling fisheries. Irrespective of this, my findings clearly showed that boldness plays a major role in selectivity of passive angling techniques at larger temporal scales. These results were further reinforced by angling experiments in **paper VI** where I compared the vulnerability of scaled and mirror carp under both, laboratory and semi-natural conditions within ponds. Independent of the environment, highly domesticated mirror carp were significantly more likely to be captured than their scaled counterparts, confirming older findings of Beukema (1969) and Raat (1985). Because mirror carp are highly domesticated fish and these fish represent the bold end of the behavioral spectrum (Huntingfort 2004; **paper II**), behavioral traits related to risk-taking and foraging activity can be considered dominant traits under selection in passive fisheries with stationary baited hooks.

All carp in my studies were raised in a common garden. Therefore, results from **paper II** and **paper VI** reveal a genetic basis of the composite trait angling vulnerability, as it has also been shown for other species (Dunham et al. 1986; Philipp et al. 2009). Compared to size and growth, the heritability of boldness and other behavioral traits is high (Mousseau and Roff 1987). Thus, under conditions of high angling exploitation and strong selection gradients acting on boldness as documented in **paper IV**, the evolutionary response of boldness-related behaviors in response to angling-induced harvest should be strong (**papers IX and X**). This should also be true for catch-and-release fisheries if cumulative hooking mortality is high. As a consequence, intensive angling fisheries should leave behind individuals that are more timid and harder to catch (**papers IX and X**). These patterns might be further reinforced by learning and phenotypic plasticity to avoid capture as I could show it for carp in **paper VI** and as is has recently been considered also for piscivorous largemouth bass (Philipp et al. 2015). This “timidity-syndrome”, defined as the emergence of fish populations that are consistently more timid when exploited compared to unexploited populations of the same genotypic background (**papers IX and X**) might have severe impacts at the population level. It is conceivable that the group performances of exploited populations decreases through removal of keystone individuals, that the decision making of these groups of fish is negatively influenced through a reduced diversity of personality types, and that the removal of bold individuals leads to leaderless groups of fish (**paper X**). Further, the ability to adapt to environmental changes, population productivity through a lack of high foraging performance, recovery after (over)exploitation, and food-web structures might all be negatively influenced by this timidity-syndrome (**papers IX and X**). Finally, a population characterized by a high fraction of timid and non-vulnerable fish should induce a mismatch between the realized catch rates and the true abundance as a large fraction of the population remains unexploited. Reductions in catch rates without corresponding declines of abundance, called “hyperdepletion” (Hilborn and Walters 1992) might have strong impacts on fisheries management and corresponding decision making as traditional surveys of fish populations will no longer reflect the current status of the fish populations, potentially leading to increasing failure of management decisions (**paper X**).

In addition to the behavioral component, angling can also select against individuals with high standard metabolic rate, maximal metabolic rate and metabolic scope as it is already known for largemouth bass (Redpath et al. 2010). In particular metabolic scope, i.e. the maximal difference between standard metabolic rate and active metabolic rate, can have severe impacts on foraging success and energy acquisition behaviors (Redpath et al. 2010). I used a subsample of N = 12 carp with known vulnerability to angling gear from the pond experiments described in **paper IV** and compared metabolic scope between vulnerable and invulnerable fish using flow-respirometry. Against findings from Redpath et al. (2010), mass-specific metabolic scope did not differ between vulnerable carp and their invulnerable counterparts in ponds (Mean ± SD = 464 ± 176 and 587 ± 198 mg O_2 kg^{-1} h^{-1} for vulnerable and invulnerable carp respectively, F = 1.39 ;P = 0.266; Fig. 6). Similarly, the genotype of the fish did not affect metabolic scope (Mean ± SD = 511 ± 266 and 535 ± 136 mg O_2 kg^{-1} h^{-1} for scaled and mirror carp respectively, F = 0.296; P = 0.598; Fig. 7) and I could not detect any relationship between metabolic scope and boldness in ponds as measured by the number of visits at the distant feeding spot (compare **papers II, IV and VI**) (Pearson`s r = -0.146, P = 0.651; Fig. 8). As summarized above, my own research on carp (**papers II, IV, and VI**) showed that boldness in the context of foraging and energy acquisition can be considered the most important trait under selection in passive angling fisheries. Based on the assumption that individuals with high metabolic needs tend to take higher risks at foraging (Biro and Stamps 2010), making them more vulnerable to angling gear as observed by Redpath et al. (2010), this result was surprising. However, findings of recent studies trying to identify correlations between basic behaviors of fish and metabolic parameters are inconsistent. Whereas several studies conducted under purely laboratory conditions were able to link risk-taking behavior and metabolic rate in numerous fish species including carp (Huntingford et al. 2010) and different salmonid species (summarized in Mathot and Dingemanse 2015), other studies conducted under natural conditions did not support these findings. For example, Farwell and McLaughlin (2009) found no correlation between standard metabolic rate and the time spent moving in juvenile brook charr (*Salvelinus fontinalis*) and Baktoft et al. (2016) were not able to correlate the metabolic rate of European perch with swimming activity in a natural lake. Including my own experiments, these inconsistent results might not only be based on the known artefacts related to solely laboratory assessments, but might also be related to insufficient incorporations of the energy management model of the study systems (Mathot and Dingemanse 2015). Because single measurements of metabolic rate- or scope are insufficient to infer the energetic constraints individual fish are faced with, limited knowledge can be derived from experiments not considering daily metabolic scopes or temporal differences in the need to acquire energy (Mathot and Dingemanse 2015). Thus, metabolic rate- and scope are likely associated with costly behaviors that bring in net energy like foraging activities under the risk of predation, but changing biotic and abiotic conditions can mask correlations between physiological and behavioral traits (Killen et al. 2013). In addition, individuals might plastically adjust only a subset of behaviors to balance their temporal energy budget (Mathot and Dingemanse 2015), making it difficult to derive definite conclusions out of my energetic experiments.

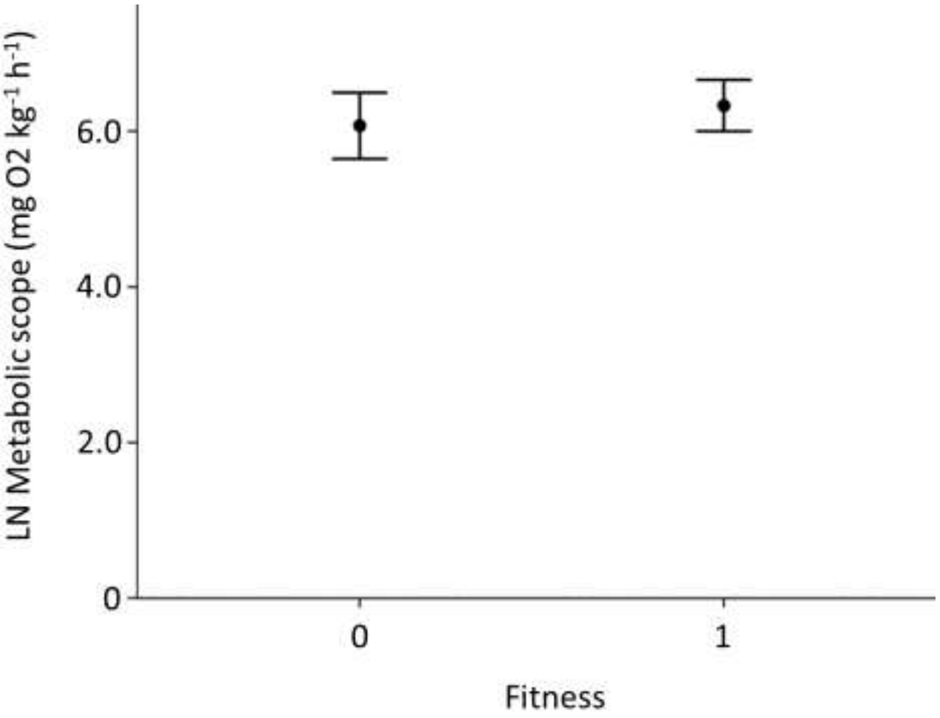

Fig. 6: Means ± SD of metabolic scope of carp previously caught (fitness = 0, N = 6) and uncaught (fitness = 1, N = 6) during experimental angling in ponds.

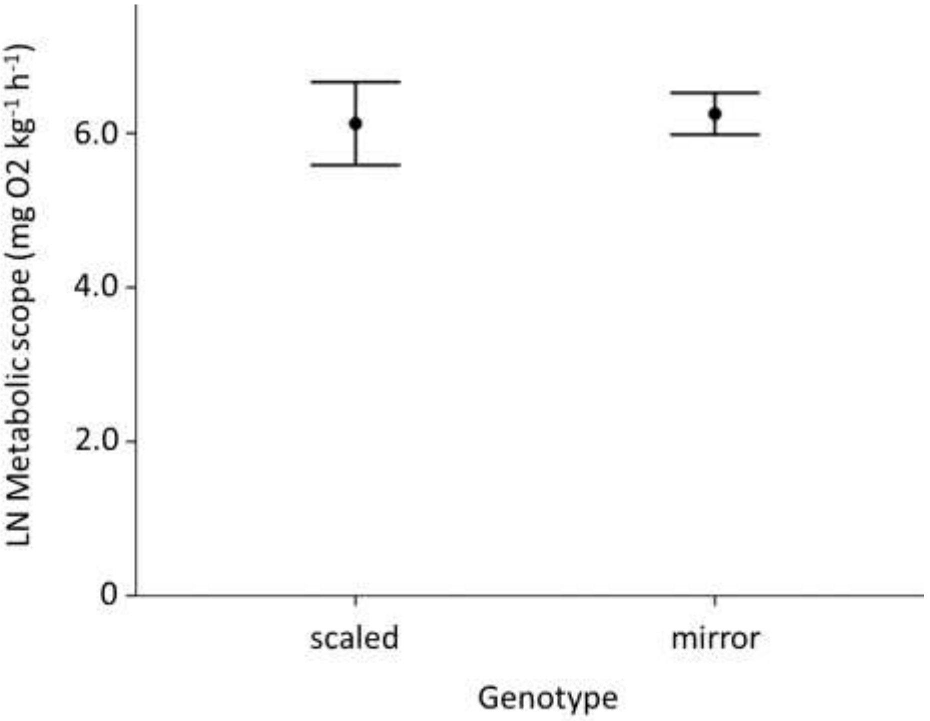

Fig. 7: Means ± SD of metabolic scope of scaled (N = 5) and mirror carp (N = 7).

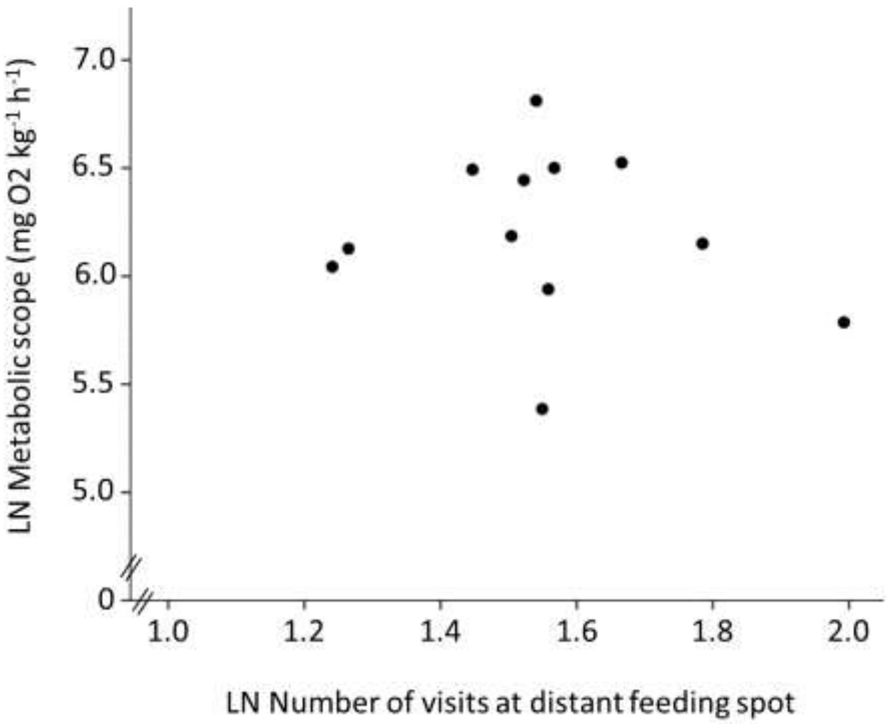

Fig. 8: Relationship between individual boldness in ponds as indicated by the number of visits at the distant feeding spot and metabolic scope.

7.3 Phenotypic correlates of individual vulnerability to angling in piscivorous fish

Similar to benthivorous carp I used omni-piscivorous amago salmon and piscivorous largemouth bass to investigate the phenotypic correlates of vulnerability to angling in two piscivorous species. As presented in **paper V**, large amago salmon were found to be more vulnerable to angling in a low pressure stream compared to their smaller conspecifics whereas in a high pressure stream no size-selectivity through angling could be observed. Moreover, catch rates were significantly lower in the high pressure stream, despite similar fish abundances within the two rivers, indicating substantial alterations of vulnerability to angling as a consequence of high fishing pressure for decades. These findings confirm recent studies in marine environments (Januchowski-Hartley et al. 2011; 2013; 2014; Alós et al. 2015a; b) and are likely based on behavioral alterations of the fish as a consequence of angling activities as described in **papers IX and X**. In difference to **papers II and VI**, plastic responses of fish could not be distinguished from genetic responses in **paper V**, but wild offspring from both rivers showed remarkable differences in boldness in the presence of a simulated predator with significantly more shy fish originating from the high pressure stream. Fish from the high pressure stream also grew less, but only when predation-risk was simulated, confirming findings from my experiments in carp where genetically based differences in boldness (and potentially correlated growth) were only expressed in stressed situations (**paper II**). In salmonids like amago salmon, angling-induced selection has been shown repeatedly to favor timid and low explorative individuals (Mezzera and Largiadèr 2001; Härkönen et al. 2014), supporting my general findings of high selection pressures acting on boldness-related traits in passive fisheries. Further, the assumption of hyperdepletion as a potential consequence of intense selective harvest (**papers IX and X**) was supported in **paper V**. Vulnerability to angling increased with the size of the fish in a low pressure environment whereas vulnerability to angling of fish from the high pressure stream initially increased with increasing size and subsequently decreased with further increases in body size albeit similar fish densities in both streams. Thus, in accordance with Alós et al. (2015b), decreasing catch rates as a result of plastic and/or genetic alterations in behavior of fished populations can be expected despite similar fish abundances, potentially safeguarding fish populations against collapse, but also lowering fishing quality and values of fisheries data as a basis for management decisions in general (**papers IX and X**).

The general findings shown above were further reinforced by experiments using the two lines of largemouth selected for high and low vulnerability in **paper VIII**. These fish showed significant differences in aggression towards hook-less artificial lures during the spawning season in ponds with high vulnerable fish hitting the lure more frequently (**paper VIII**). This result is in line with findings from Cooke et al. (2007) who angled fish from the same selection lines off their nests and found high vulnerable fish to be captured after 1.6 ± 0.2 casts, whereas low vulnerable fish needed 4.3 ± 0.9 casts to get hooked. It can be assumed that these differences in vulnerability between the two selected lines are mainly based on aggression rather than boldness, because Binder et al. (2012) found similar swimming activities for the two selection lines of fish and Nannini et al. (2011) found higher foraging efficiencies for low vulnerable fish. Thus, traits usually correlated with boldness did not differ between the two selected lines of piscivorous largemouth bass or were inversely related compared to expectations when assuming boldness to be the main predictor of angling vulnerability. However, depending on the lure used, angling on largemouth bass can also be selective along the shy-bold continuum (Wilson et al. 2015) with more nature-like lures targeting the shy end of the vulnerable population. All together my results indicate that there are not only differences in behavior between vulnerable and invulnerable fish (**papers IV, V, VI and VIII**), but that there are also differences in behavioral traits determining the vulnerability of benthivorous and piscivorous species. Whereas in benthivorous carp and omni-piscivorous amago salmon caught with natural baits, angling mainly selects against boldness in the context of energy-acquisition, vulnerability of solely piscivorous species like largemouth bass seems to be largely dependent on aggression.

7.4 The mechanistic basis of vulnerability to angling

The main findings of **paper IV** already indicate the paramount importance of boldness in explaining individual vulnerability towards angling gear in carp. More individual characteristics of fish likely attribute to the complex trait of angling-vulnerability (compare Fig. 1). From a mechanistic perspective, individual- and social learning abilities (Beukema 1970; Raat 1985), food ingestion rates (Matsuzaki et al. 2009; Nannini et al. 2011), food preferences (Suzuki et al. 1978) and environmental conditions in combination with genotype x environment interactions (**paper II**) may impact behavior and therefore vulnerability of fish. In **paper VI** I found mirror carp to be significantly more active during foraging, faster in ingesting the first food item, and mirror carp also ingested a higher total number of food items within replicated trials. These findings from **paper VI** mechanistically show that intrinsically high foraging activity- and speed likely explains why explicitly bold carp are caught more often even if several fish are within close vicinity of the baited hook as it was the case during experiments in **paper IV**. Albeit carp in **paper VI** showed clear signs of individual and social learning abilities as indicated by declining catch rates over time and increasing visual and tactile hook avoidance, these effects did not differ between scaled and mirror carp. Similarly, food preferences did not differ between the two genotypes, again highlighting boldness and underlying traits like intrinsic foraging activity- and speed to be the main factors determining individual vulnerability of carp in passive angling fisheries.

A certain fraction of carp was completely invulnerable to capture (45% and 15% of mirror carp and 68% and 45% of scaled carp within ponds and the laboratory, respectively) in **paper VI** and relatively more fish of both genotypes were caught within the laboratory. These findings are comparable to Beukema (1969) who found 18% of domesticated mirror carp and 33% of less domesticated scaled carp to be invulnerable to angling. However, the differences in catch rate between the two environments in **paper VI** also indicate environmental factors to significantly impact vulnerability of fish. Based on **paper VII** where experimental pike fishing in the natural Döllnsee was conducted, I could identify five environmental parameters that significantly impacted capture success. The catch rate was dependent on the past two days fishing effort, time of the day, average daily water temperature, wind speed, and moon phase, together explaining about 21.4% of the variability in catch per unit effort. The past two days fishing effort affected capture success of anglers much more than abiotic or meteorological variables, indicating a short-term behavioral response of fish towards angling activities or cumulative responses towards catch and release events (Arlinghaus et al. 2008; Klefoth et al. 2008; 2011; Baktoft et al. 2013). Comparable to my findings in **paper VI**, learned hook avoidance with increasing fishing time might have added to this finding, although vulnerability of pike was only impacted two days back, the test environment was much larger and no sudden drop of capture success could be observed as it has been reported in other studies (Raat 1985; van Poorten and Post 2005; Askey et al. 2006). Irrespective of species my findings show that the composite trait vulnerability to angling is not solely dependent on the behavioral expressions of the individual fish, but also on environmental factors, angler behavior, learning abilities and genotype x environment interactions, potentially influencing selection strength on phenotypic expressions in dependence of the environment and its complexity.

7.5 Fitness consequences of angling-induced selection

It is generally assumed that natural predation of juvenile fish will favor relatively slow growing and timid individuals so that a directional selection against boldness and growth can be assumed under both, natural and artificial selection (Edeline et al. 2007; 2009). In my additional experiments on carp I experimentally tested the hypothesis that natural selection of juvenile fish points into the same direction than artificial selection through angling. I used carp with known vulnerability to angling from **papers IV and VI** and stocked them into replicated ponds with large pike and two control ponds without pike over winter and additionally stocked them into the natural Döllnsee which is known to have a high density of predatory pike (Kobler et al. 2008). In both cases survival of the fish was measured.

Within the ponds natural mortality without pike predation was 14% whereas natural mortality in the vicinity of pike predation was 65%. In agreement with the hypothesis, the likelihood of mortality was significantly higher for juvenile carp that have been vulnerable to angling gear in previous experiments and also for smaller fish, independent of vulnerability (Tab. 1; Fig. 9).

Tab 1: Generalized linear mixed model with pike predator nested within ponds as a random factor to determine survival fitness of vulnerable and angling-invulnerable carp towards natural predation-risk.

Variable	Coefficient	SE	Z-value	P	Pseudo R^2 **0.33**
Intercept	4.11	2.77	1.49	0.138	
Captured (yes)	1.04	0.51	2.07	**0.039**	
TL (mm)	-0.03	0.01	-1.83	0.068	

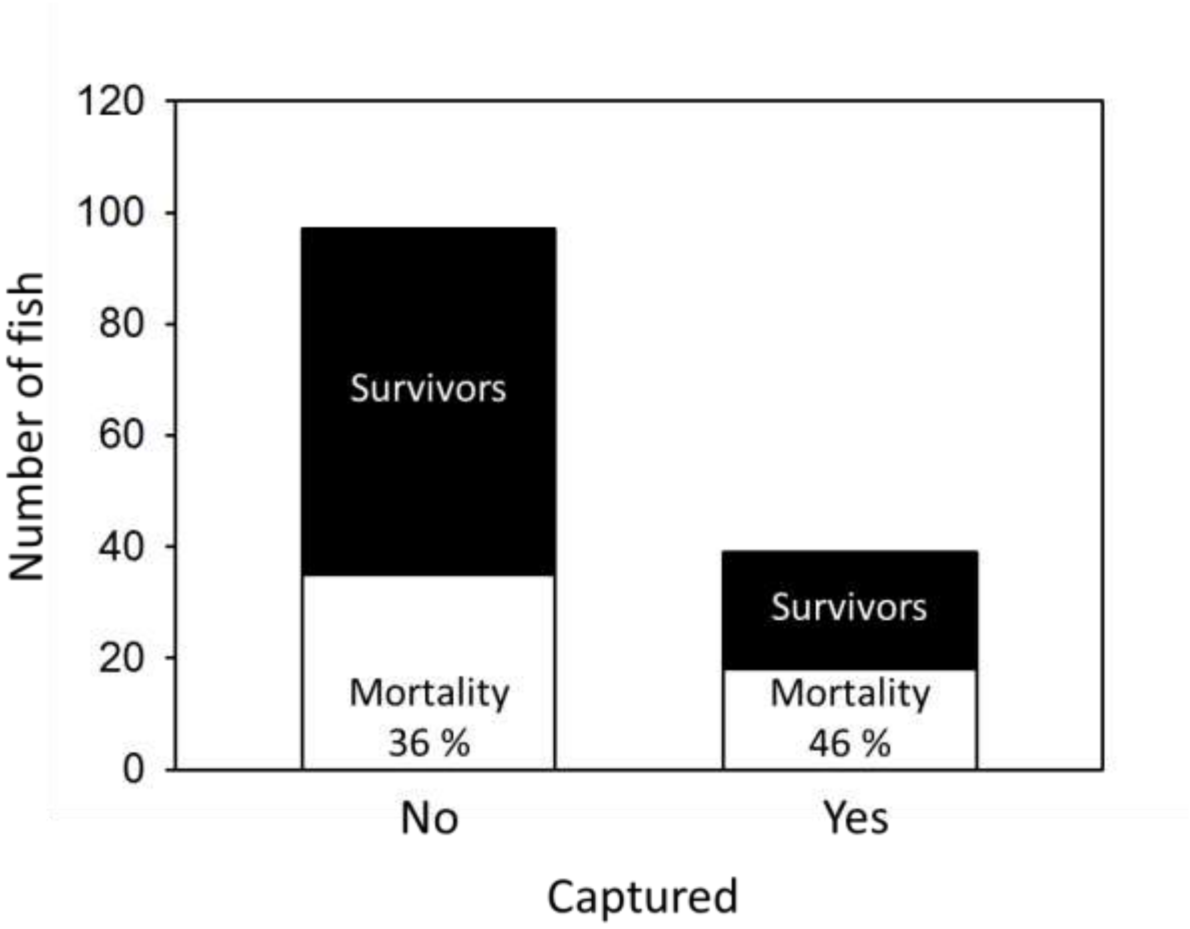

Fig. 9: Relative survival probability of previously angling-caught and angling-uncaught carp in the vicinity of a natural pike predator in ponds.

Similarly, survival probability of juvenile carp stocked into the natural Döllnsee was higher for fish previously invulnerable to angling in ponds. Mean survival time between previously caught and uncaught fish strongly differed (mean survival days in Döllnsee of previously caught and uncaught fish ± SD 7.8 ± 7.5 and 174.6 ± 210.1; range 1 – 25 and 1 – 400, respectively), although this finding was not significant, probably as a result of low sample size (N = 16; ANOVA, df = 1, F = 2.47, P = 0.139). Whether mortality always occurred as a consequence of predation by predatory fish is unknown, but during the experiment two pike were caught by angling with a transmitter of carp in their stomach. It can therefore be assumed that natural predation of pike caused a majority of mortalities.

My findings from the two experiments under both, replicated semi-natural conditions in ponds and fully natural conditions in Döllnsee were in line with the hypothesis that angling induced selection and natural selection in juvenile fish point into the same direction. Thus, angling can be seen as additional predation-risk within a high-risk environment where explicitly bold and fast growing individuals show the lowest survival probability. However, it can also be assumed that after reaching maturation additional fitness components like reproductive success override survival as superior fitness component (Edeline et al. 2007) and in such cases, angling-induced selection might act against natural selection. To test this hypothesis in adult fish, experiments on nest-guarding largemouth bass were conducted in **paper VIII**. Using males from two lines of largemouth bass selectively bred over three generations for either high (HV) or low (LV) vulnerability to angling as a model system, we could show that the composite trait “vulnerability to angling” positively correlated with aggression, intensity of parental care, and reproductive fitness. HV males spent relatively and absolute more time guarding their nests and fanning their eggs than did LV fish and they showed higher aggression towards potential brood predators that were simulated by hookless fishing lures. Similarly, larger fish from both groups showed higher parental care intensities. The selected line and size of the male had a significant interactive influence on individual reproductive success in terms of offspring number where large HV males produced most offspring (**paper VIII**). Thus, when anglers target the largest and fittest fish, angling induced selection can have negative consequences for reproductive success in wild populations of largemouth bass and potentially other species where behaviors like aggression and nest-guarding intensity not only determine vulnerability to angling but also reproductive fitness.

My results from **papers IV, V, VI and VIII,** and my additional fitness experiments collectively showed that 1) angling on piscivorous largemouth bass and potentially other species with a comparable life-history selectively targets those individuals that exhibit the highest reproductive fitness potential, 2) angling selects against behavioral traits that determine vulnerability and reproductive success like aggression in nest-guarding species, 3) angling selects against bold and fast growing individuals with high foraging activities in benthivorous and omni-piscivorous species, potentially causing future reductions of fecundity based on impaired growth rates and direct selection against large and fast-growing individuals at the adult stage, 4) in juvenile benthivorous fish like carp, natural selection and angling-induced selection point into the same direction whereas at an adult stage selection probably acts against natural selection, 5) independent of the species angling-induced adaptive changes either based on evolution or plasticity can lower general vulnerability of fish stocks even at constant fish densities, and 6) intensive catch-and-kill type angling not only alters age and size distributions within a population, but can also disrupt the generally positive relationship between body size and vulnerability to angling gear.

From an evolutionary perspective (as described in **papers I, IX and X**), fish populations irrespective of species and life-history will evolve towards timidity under high fishing pressure because bold, aggressive and fast-growing individuals are harvested. This result is in line with theoretical expectations (**papers I, IX and X**) and recent studies on marine fish species (Alós et al. 2015a; b), all showing a timidity-syndrome as a consequence of human-induced predation-risk. Following this framework, exploitation of fish through angling will cause plastic and evolutionary behavioral responses with increasing timidity and flight initiation distances. In this landscape of fear (Laundré et al. 2014) lethal harvest and/or unwanted catch-and-release mortalities will adaptively impact life-history and behavioral traits, collectively shaping the timidity-syndrome. It currently remains open if the timidity-syndrome is of short duration and fish will only change their behavior in direct response to an angling event or if affects are much longer lasting (**papers IX and X**). However, based on the work of Philipp et al. (2009), Alós et al. (2015a; b) and my own research in **paper V**, longer lasting and evolutionary effects can be considered a real scenario, potentially also influencing the behavior of fish towards their natural predators. Based on increasing timidity along the food-web, influencing both bottom-up and top-down processes, even complete ecosystem functioning might be altered (Laundré et al. 2014, **papers IX and X**).

My research showed that in juvenile fish angling-induced selection can strengthen natural selection, whereas in adult fish angling-induced selection can act against natural selection. Thus, being too bold in a juvenile stage will decrease survival fitness, irrespective of fishing activities, whereas being bold as an adult can increase reproductive fitness, but angling-induced selection acts against natural selection in this life-stage. Consequently and depending on the ecological context, anglers can negatively impact recruitment within wild populations through directional selection of behavior when behavioral determinants of angling vulnerability like boldness and aggression also predict reproductive success. Alternatively to this directional approach, fisheries-induced selection on traits like boldness and growth might also turn disruptive as a consequence of interplays between natural selection and harvest activities (Carlson et al. 2007; Edeline et al. 2007; 2009), i.e. when natural selection and fishing-induced selection on adult fish strongly act in opposite directions (Edeline et al. 2009). This approach, however, remains hypothetical and might also depend on foraging opportunities (Edeline et al. 2007; 2009). Under non-restricted food conditions, formerly shy fish might increase their reproductive fitness expectations as an adult through increasing bold behavior aiming to gather more food and to grow faster. This way, the strength of opposing natural- and fishing-induced selection pressures might be relaxed through flexible plastic responses of the individual fish towards the fitness landscape. The strategy of being shy as a juvenile and being bold as an adult might pay-off under non-restricted food conditions whereas no such plastic response might be promising when food is rare, because grow expectations would be comparably small whereas survival expectations remain low, depending on the fishing pressure. Thus, angling-induced selection should usually act directional against bold and aggressive behavior and disruptive or other selection responses might occur in extreme cases where natural selection and fishing-induced selection strongly act in opposite directions and plastic responses of the fish cannot balance disruption.

Another alternative perception could be based on the links between behavior, metabolism and growth. As shown in **paper IV**, bold behavior can positively influence growth rate in carp and consequently, fish might grow less if the bold individuals are selected by anglers. However, in largemouth bass angling exhibits a decrease in metabolic rate (Redpath et al. 2010; Hessenauer et al. 2015) and under food-restricted conditions, these low vulnerable fish with low metabolic rate might even grow better (Redpath et al. 2010). As a consequence and alternatively to the above, one can also predict that fisheries-induced evolution of timidity without a necessary response in growth can be a likely outcome in many situations (compare **paper X**). In this case fitness impairments through selective harvest might be lowered, but more research is needed to disentangle the relationships between behavioral selection and population dynamics.

8. Conclusions

The objective of my doctoral research was to disentangle the phenotypic correlates of individual vulnerability to angling. Based on comprehensive phenotypic descriptions covering several behavioral-, life-history-, morphological, and physiological traits, I identified the potential for angling-induced evolutionary changes. In addition I revealed that phenotypic variability within fish populations might add to the conservation of populations under fished conditions. The main conclusions of my research can be summarized as follows:

1. The environment used to properly evaluate behavioral correlates of vulnerability to angling must be based on real field conditions or must reflect nature-like conditions including explicit predation-risk. Otherwise study outcomes can be biased

2. Boldness in the context of foraging is the most important trait under selection in passive fisheries targeting benthivorous species whereas aggression determines selection in piscivorous species.

3. The mechanistic basis of vulnerability to angling is based on an interplay of species, individual behavior, learning and environmental factors.

4. Natural selection and angling-induced selection point into the same direction in juvenile fish whereas angling-induced selection can severely impact reproductive fitness when behavioral patterns that determine fitness, like aggression in nest-guarding species, also affect their vulnerability to angling gear.

I identified boldness in the context of foraging to be the most important factor in determining vulnerability of benthivorous carp whereas aggression can be considered to be the most important trait in piscivorous largemouth bass. Based on similarities in life-history and behavior of many benthivorous and piscivorous fish, these results can likely be transferred to many other species. As a consequence of my findings, an exploitation-induced timidity syndrome can be assumed in highly exploited fish stocks leading to increasing shyness and an overall reduced vulnerability of the individual fish with negative consequences for the number of trophy fish, management decision making based on poor data and angler satisfaction in general.

In the future and to better understand the consequences of timidity-syndromes, fish populations should be routinely monitored for changes in boldness and other behavioral characteristics over time. In clear water conditions, camera systems might help to identify behavioral changes as a consequence of selective harvest. Alternatively, novel tracking systems like CDMA technology, phenotypic correlates of behavior like morphological characteristics or the development of functioning and standardized rapid behavioral assays under laboratory conditions can potentially be used for this purpose.

9. Management implications

From a management perspective my findings call for a promotion of behavioral diversity. This may be achieved through reduced harvest, relocation or stocking of naïve fish or through well designed protected areas where highly vulnerable phenotypes can migrate into the fished areas, thereby obtaining phenotypic diversity. All of these management tools will differ in their applicability and functionality and based on the managed ecosystem, managers will be faced with various trade-off decisions. For example, many fished freshwater ecosystems in Germany are small and not connected, casting protected areas into doubt as many ecosystems are simply not big enough to establish a management scenario comparable to the common marine protected areas. Further, the potentially easiest and best solution, a general reduction of harvest through intensified release of large, bold, and fast-growing individuals is often hampered by legal concerns in Germany. Thus, alternatives like fish relocation or stocking of domesticated and highly vulnerable individuals might be a better choice to maintain angler satisfaction, but potential negative effects of unwanted gene transfer, reduced reproductive ability, and high mortality rates cannot be ruled out. Finally, aquaculture companies might develop a market where wild-like fish originating from natural lake and pond-ecosystems are increasingly produced as stocking material. This would mean a change of common aquaculture because the ponds would need to consist of diverse species and habitats to maintain phenotypic diversity and parental fish for reproduction would ideally origin from the stocked water body. After draining, these fish from all size classes could be simply transferred to the fished water-body, comparable to a translocation experiment. Because I am currently working as a fisheries manager, I will try to transfer my own research results directly into practice, aiming to establish biologically sustainable fisheries.

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Declaration of authorship

I do hereby solemnly declare that I have completed the preceding PhD thesis independently, and have not used any other sources or aids apart from those listed.

Hiermit erkläre ich, die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt zu haben.

Hannover, 27.09.2016

Thomas Klefoth

Appendices

Paper I

I

Silva Uusi-Heikkilä, Christian Wolter, Thomas Klefoth, Robert Arlinghaus, 2008. A behavioral perspective on fishing-induced evolution. Trends in Ecology & Evolution, 23:419-421.

A behavioral perspective on fishing-induced evolution

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The potential for excessive and/or selective fishing to act as an evolutionary force has been emphasized recently. However, most studies have focused on evolution of life-history traits in response to size-selective harvesting. Here we draw attention to fishing-induced evolution of behavioral and underlying physiological traits. We contend that fishing-induced selection directly acting on behavioral rather than on life-history traits *per se* can be expected in all fisheries that operate with passive gears such as trapping, angling and gill-netting. Recent artificial selection experiments in the nest-guarding largemouth bass *Micropterus salmoides* suggest that fishing-induced evolution of behavioral traits that reduce exposure to fishing gear might be maladaptive, potentially reducing natural recruitment. To improve understanding and management of fisheries-induced evolution, we encourage greater application of methods from behavioral ecology, physiological ecology and behavioral genetics.

The potential for fishing-induced evolution (FIE) has been discussed recently [1,2]. Most studies reviewed in Ref. [1] have focused on life-history traits that directly or indirectly determine body size. Under the common scenario of size-selective harvesting, large fish face a fitness disadvantage that might cause rapid evolution toward earlier maturation at smaller sizes, higher reproductive investment and lower intrinsic growth capacity and, collectively, smaller size-at-age [2]. Such evolution can degrade fisheries yield and other ecological services within decades [2].

Many studies on FIE, however, fall short in addressing the selection pathways that drive the observed life-history changes. For example, evolution of small body size can result from direct selection for decreased intrinsic growth capacity or be a consequence of selection on correlated life-history or behavioral traits [3]. Indeed, in some passively operated fishing gears (e.g. trapping, angling, gill-netting), behavioral traits rather than body size *per se* determine a fish's vulnerability to capture, and thus its survival and fitness (Figure 1) [3]. In these situations, direct selection on behavior can drive evolutionary changes in correlated life-history traits such as growth rate [3] because the more active, bold and vulnerable individuals tend to also grow faster [4,5]. Despite the important role of behavior in influencing catchability in various fisheries [3,6–8], the behavioral dimension of FIE has largely been neglected.

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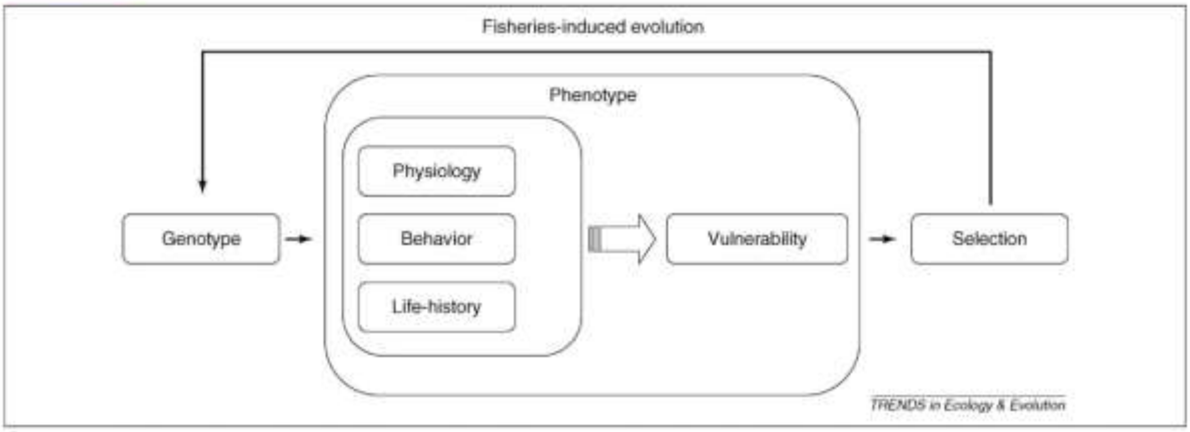

TRENDS in Ecology & Evolution

Figure 1. Mechanistic pathway of fishing-induced evolution by selection on fishing vulnerability. In this scheme, vulnerability to capture is considered a heritable trait as part of the fish's phenotype. Vulnerability to capture comprises a bundle of physiological, behavioral and life-history traits that jointly determine vulnerability to capture. In passive fisheries, vulnerability to capture is largely determined by specific behavioral patterns rather than by body size-related life-history traits *per se*. Due to genetic correlations between behavioral, physiological and life-history traits, fisheries-induced selection on behavioral traits might alter physiologies and life histories, but behavior might also change in response to selection on correlated life-history or other traits.

Evolutionary responses to fisheries-induced selection depend on the selection differential and the heritability of the trait [1]. Large selection pressures on behavioral traits can be expected when specific behavioral patterns increase the encounter probability with the fishing gear, thus influencing survival and fitness. For example, vulnerability to capture by gill-nets not only depends on body size and shape but is also strongly related to an individual's activity level [3,7]. Similarly, in recreational angling, vulnerability to capture can be size related, but most importantly depends on a fish's decision to attack and/or ingest baited hooks [7–9]. In this context, bold and aggressive personalities, individuals with lower cognitive abilities and those with higher metabolism and growth capacity often take more risks and hide less in structured habitat, rendering these fish more vulnerable to capture [3,7,8]. Thus, behavior-driven vulnerability to fishing might constitute an underappreciated mechanism for selection on growth rate [3] or other life-history traits [5]. Alternatively, due to genetic correlations between physiological, behavioral and life-history traits (Figure 1), evolution of behavioral traits might be an indirect consequence of selection on body size under strongly size-selective harvesting. Collectively, if exploitation directly or indirectly induces a large selection differential on particular heritable behavioral traits, evolving fish stocks will not only become less abundant and smaller [2] but also harder to catch [3,6–9], which diminishes the quality of the fishery.

Selection responses of behavioral traits to fishing can be rapid because heritabilities of behavioral traits are often larger than those of life-history traits [10,11]. Indeed, in largemouth bass (*Micropterus salmoides*), artificial selection for vulnerability to recreational angling induced evolutionary changes in various physiological and behavioral traits after only four generations [8]. Vulnerable individuals had higher metabolic rates and resting cardiac activity, and provided more intense parental care than invulnerable fish of the same body size [8]. Vulnerability to capture therefore was primarily determined by physiological and behavioral traits rather than by body size. This suggests that selective harvest of highly vulnerable largemouth bass could impact the population in the long term by altering parental care activity and level of aggression [8]. Moreover, in nest-guarding species, FIE is conceivable even in the absence of fishing mortality, for example when recreational anglers practice catch-and-release during the reproductive period [8]. In these situations, the fitness of more aggressive and vulnerable individuals is reduced when they are temporarily removed from their nests, leaving the brood susceptible to rapid egg predation [12]. Over time, this might favor more wary and less vulnerable genotypes that happen to also be inferior nest guarders.

The potential for evolution of behavioral and physiological traits and its consequences for life history, demography and fishing quality constitutes a fascinating, yet largely overlooked research area within the emerging field of FIE. To improve understanding and management of FIE, we encourage collaboration between fishery scientists and evolutionary ecologists (*sensu* [1]), and greater application of methods from behavioral ecology, physiological ecology and behavioral genetics.

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Paper II

II

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ORIGINAL PAPER

The role of ecological context and predation risk-stimuli in revealing the true picture about the genetic basis of boldness evolution in fish

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Abstract To showcase the importance of genotype × environment interactions and the presence of predation risk in the experimental assessment of boldness in fish, we investigated boldness in terms of feeding behavior and refuge use in two genetically different populations of juvenile carp (*Cyprinus carpio*) in two replicated experimental conditions in ponds and laboratory tanks. The populations were expected to exhibit genetic differences in boldness due to differential evolutionary adaptation to low-predation-risk pond aquaculture conditions. Boldness was measured in variants of open-field trials with and without implementation of additional predation risk-stimuli by angling on feeding spots. Without explicit implementation of risk, genotypes adapted to low-risk environments, i.e., domesticated mirror carp behaved consistently bolder than their less domesticated scaled conspecifics in the pond environment, but not in the laboratory environment. When we implemented artificial risk-stimuli by angling on previously safe feeding spots, boldness differences among genotypes also emerged in the laboratory environment, indicating strong genotype × environment effects on boldness behavior of carp. The expected genetic basis of boldness differences among genotypes was clearly supported in the pond environment, while the laboratory study revealed these patterns only under inclusion of explicit risk-stimuli. Our study thus underscores that boldness may involve both a basal component that is expressed independently of obvious predation risk (e.g., in open fields) and a component revealed in relation to explicit predation risk, and both dimensions may respond differently in behavioral tests.

Keywords Genotype × environment interactions · *Cyprinus carpio* · Predation risk · Common garden · Angling

Introduction

Evolutionary adaptation of life-history traits in response to predation-induced selection pressures is well documented in several taxa ranging from insects, over birds, and fish (Sæther 1988; Reznick et al. 1990; Gotthard et al. 1994). It has also been commonly reported that behavioral traits vary consistently within and between animal populations in response to the level of predation risk (Seghers 1974; Cousyn et al. 2001; Stoks et al. 2003; Ghalambor et al. 2004; Herczeg et al. 2009). Such adaptation includes behavioral traits commonly summed under the temperament trait "boldness" (Herczeg et al. 2009). In fish, boldness—defined as the individual's reaction to any risky, but not new situation (Réale et al. 2007)—is expressed in behaviors like use of risky habitats (Wilson and McLaughlin 2007), exploration and activity (Wilson and Godin 2009), foraging under risk of predation (Wilson and Stevens 2005), or schooling (Seghers 1974). In line with the hypothesis that boldness-related traits should evolve in response to predation-induced selection pressures (Seghers 1974), populations of fish adapted to low-predation-risk conditions were found to exhibit greater

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risk-taking behavior than fish adapted to high-risk conditions (e.g., Seghers 1974; Magurran et al. 1992; O'Steen et al. 2002; Ghalambor et al. 2004). The main explanation put forward for these findings is that too bold behavior can be disadvantageous in the presence of predators by increasing the probability of deadly attacks, such that the average boldness of a population of fish should be lower in high-predation environments relative to low-predation conditions (Seghers 1974; Brydges et al. 2008).

Studies on genetic adaptation of behavioral traits can be challenging and have mainly been pursued by a comparative approach where populations supposed to be adapted to different predatory regimes have been compared, often using fish as a model species (Brydges et al. 2008; Conrad et al. 2011). When properly conducted, differences in average boldness among populations adapted to different levels of predation risk can support inferences about an underlying genetic basis of behavioral phenotypes. Such inferences are particularly strong if examinations of adaptation of behavioral patterns to predation risk are conducted using common-garden reared animals under laboratory conditions (Kawecki and Ebert 2004). Indeed, many studies on boldness differences among populations of fish have used common-garden reared offspring and subsequently applied laboratory-based boldness assessments (e.g., O'Steen et al. 2002; Herczeg et al. 2009). Some comparative studies on boldness differences among fish populations used individuals directly collected in the wild (Magnhagen 2006; Archard and Braithwaite 2011). These studies reported that fish from high-predation-risk environments exhibited greater (rather than lower) risk-taking behavior compared to individuals collected from low-predation-risk environments (e.g., Brown et al. 2005; Magnhagen 2006; Archard and Braithwaite 2011). However, in the absence of common-garden designs, study findings may well be explained by plasticity rather than genetically based evolutionary adaptation (Conover 1998; Kawecki and Ebert 2004).

Common-garden studies can provide stronger inferences about the potential genetic basis of behaviors, but such studies are not free of biases when behavioral assays are conducted in laboratory contexts (Nuismer and Gandon 2008). This is due to uncontrolled effects of the artificial assessment environment on the test animals and their phenotypic expressions (Kawecki and Ebert 2004). A range of laboratory effects may potentially explain conflicting findings in earlier among-fish population comparisons in terms of boldness-related behaviors. For example, Bell (2005) failed to identify expected differences in swimming activity outside refuges between common-garden reared offspring of three-spined stickleback (*Gasterosteus aculeatus*) populations with different predation backgrounds, when trials were conducted in a novel laboratory environment that lacked explicit predation-stimuli. By contrast and being consistent with expectations, the fish supposed to be genetically adapted to high predation risk were indeed found to be more timid than those adapted to low-risk conditions when observed in the presence of a predator. Although not specifically discussed by Bell (2005), this study highlights the potential for genotype × environment interactions in experiments when behavioral responses of genetically different animals across various environments or situations are observed. Moreover, in behavioral tests, different components of genetic adaptation of the complex trait "boldness" might be measured, and any subdimensions of the supposed overarching boldness construct may have evolved different responses in relation to predators. For example, in the population studied by Bell (2005), local behavioral adaptation to predation risk might not have happened on the basal level of behavior (e.g., swimming activity in the absence of obvious predation risk), but rather on the behavioral response to predation risk, which would subsequently only be expressed under test conditions including risk-stimuli. Consequently, behavioral phenotypes revealed in experimental trials by fish may be strongly affected by genotype × environment interactions and the presence or absence of predation-stimuli, highlighting the importance of standardized experimental setups when researchers aim to identify phenotypic differences between differently adapted populations. Otherwise, study findings, particularly regarding the genetic basis of observed behavioral differences and generality of these findings, need to be treated with caution.

In a second example on the difficult issue of inferring the genetic origin and the exact portion of the boldness axis revealed through laboratory experiments on comparative boldness differences among fish populations, Brown et al. (2007) found laboratory-reared *Brachyraphis episcope* derived from parents from high-predation sites to emerge significantly faster from a shelter than lab-reared fish derived from low-predation parents. These results were derived using classical open-field tests designed to neutrally measure boldness in fish. Following common expectations (Seghers 1974), too bold behavior should have been outselected under high predation risk such that one would have expected fish from high-predation sites to emerge more slowly from shelter compared to fish from low-predation sites. Yet, no predation risk-stimuli were implemented during the open-field tests by Brown et al. (2007). It is thus unclear whether the basal boldness expressed by the study animals in the open field would have been different in the presence of more explicit predation risk-stimuli as highlighted by the study by Bell (2005). Indeed, the neutral open-field test within the laboratory, as applied by Brown et al. (2007), was only designed to reveal basal differences in boldness and did not aim at testing alternative traits under selection such as predator recognition or response to explicit predation risk. Because all of these traits together are characteristic for the complex composite trait "boldness," the true underlying differences in boldness among populations should ideally be tested to cover a greater range of plausible behavioral reactions of fish exposed to varying predation regimes in the wild. Our examples highlight the necessity for explicitly accounting for the impact of predation risk-stimuli to isolate the effects of the genotype, the environment, and genotype × environment interactions on boldness-related behavioral comparisons among fish populations that are supposed to be evolutionarily (i.e., genetically) adapted to predation risk.

To elucidate the impact of the assessment environment (pond or laboratory environment) along with the inclusion of predation risk-stimuli on among-population differences in boldness, in the present study, we compared the expression of boldness-related traits among two juvenile carp (*Cyprinus carpio* L.) genotypes reared in common-garden prior to experimentation. The two populations were differentially adapted to low-predation-risk pond aquaculture conditions and should therefore differ in average boldness. This is because farmed fish have been consistently found to be bolder relative to less domesticated fish (Berejikian 1995; Huntingford 2004; Huntingford and Adams 2005; Conrad and Sih 2009). Therefore, we would expect our carp populations to consistently differ in average boldness in an open-field test in the laboratory and in the more natural pond environment, both in the basal boldness as well as in their response to explicit risk of predation.

Material and methods

Our experiment was designed to measure how two genotypes of common-garden reared carp with known differences in adaptation to predation risk differ in their expression of three boldness-related traits (number of visits at two different feeding spots and intensity of sheltering) in a non-novel environment and to assess whether there is an impact of the assessment environment and of artificially induced predation risk-stimuli on boldness expressions of the fish within the different environments. First, behavior of the fish was tested in three replicated ponds. The pond environment did not contain any natural fish predators, but offered latent predation risk through fish-eating birds and potentially through olfactory cues from predators like pike (*Esox lucius*), because the ponds were continuously supplied with water from a large natural lake with known existence of several fish predators (Lewin et al. 2004). Moreover, the ponds represented an environment comparable to the original evolutionary environment of the test fish (e.g., farm ponds used for aquaculture). In a second step, the same boldness-related traits were measured in a situation where artificially induced predation risk-stimuli were implemented through standardized angling on feeding spots to test for the effects of artificial risk-stimuli on boldness expressions of the study fish. To further investigate the role of risk-stimuli for the expression of adapted differences in boldness and further to remove any potentially confounding factors that might have existed in the outdoor ponds, similar replicated experiments were conducted within a large laboratory tank without any kind of predation risk-stimuli except for the standardized angling tests. Again, fish were first observed without any risk-stimuli, followed by observations during implementation of risk. In this way, we were able to experimentally test for the effects of the assessment environment (semi-natural in ponds vs. laboratory) and the effects of artificially induced predation risk-stimuli (i.e., angling on feeding spots) within the two different environments on boldness expressions of fish with known differences in adaptation to risk.

Study animals

Among fish, pond-cultured carp exhibit the longest history of artificial selection starting about 2,000 years ago (Balon 2004). Distinct carp genotypes and phenotypes have developed as a consequence of adaptation to suites of low-predation-risk pond conditions (Steffens 1980; Balon 2004). The most obvious phenotype indicating differential degree of artificial selection and adaptation to pond environments is the scale pattern of common carp, which can be broadly distinguished into scaled and mirror phenotypes (Balon 1995). Scaled carp are fully scaled, reflecting the original morphotype of wild common carp, whereas mirror carp have much less scales, reflecting the morphotype that is strongly domesticated and highly adapted to low-risk pond conditions (Probst 1953; Balon 2004; Matsuzaki et al. 2009). All carp used in our study were raised at a commercial fish hatchery (Fischzucht Wegert, Ostercappeln, Germany; 52°19′52″ N, 8°14′48″ E) in the same common-garden pond environment. Parental fish descended from two selection lines: (1) a selection line with scaled morphotypes and (2) a selection line in which scaled morphotypes were previously crossed with strongly domesticated mirror carp selection lines. Fish from both selection lines were stocked into the same common-garden pond for reproduction. Young-of-the-year mirror carp could only develop as a result of two breeders from the strain originally crossed with domesticated mirror carp (strain 2) (Kirpichnikov and Billard 1999). All juvenile carp were exclusively fed with standard carp dry food in addition to any natural food ingested in the shallow (1.5 m deep) earthen common-garden pond (40 m × 50 m). At an age of 10 months, the fish were transported to the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin, Germany. There, fish were kept in tanks (1 m × 1 m × 1 m; 5 fish per 100 l) with tap water (mean temperature±SD 18±1.5°C, exchange rate once per day). Fish were fed with standard carp pellets (5 mm diameter, Aller Classic, Aller Aqua, Golßen, Germany), and the total daily food amount was ~1.5% of the fish body wet mass. Before the behavioral experiments started, fish were slowly acclimatized to water temperatures within the test environments (see the following discussion) by altering the temperature at a maximum of 1°C per day.

Tagging of fish

All carp ($N=100$ scaled carp and $N=100$ mirror carp) were individually marked with passive integrated transponders (PIT) to observe fish behavior using PIT antenna systems. We surgically implanted PIT tags (23 mm length, 2 mm width, Oregon RFID, OR, USA, 2% tagging mortality) into the fish's body cavity following the method described by Skov et al. (2005). Before PIT implantation, fish were anesthetized using 1 ml l^{-1} of 9:1 solution of ethanol:clove oil in well aerated water at 18°C. After PIT implantation, all fish were measured for total length (TL, to the nearest 1 mm) and wet weight (to the nearest 1 g).

Behavioral experiments under pond conditions

Stationary passive telemetry systems within three replicated experimental ponds (12 m×5 m×1 m; $L \times W \times H$; Fig. 1) were simultaneously used to enumerate carp behavior by two genotypes in ponds in September 2008. To investigate boldness parameters, each of the three ponds was stocked with 40 similar-sized carp (20 scaled carp and 20 mirror carp, mean TL ± SD pond 1: 199±6.9 and 199±12.1 mm, *T*-Test, $t=0.08$, $P=0.936$; pond 2: 199±4.7 and 200±11.9 mm, *T*-Test, $t=-0.26$, $P=0.797$; pond 3: 199±6.1 and 197±11.2 mm, *T*-Test, $t=0.78$, $P=0.440$), which were allowed to acclimatize for 2 days before behavioral observations started.

The ponds were continuously supplied with water from the nearby Müggelsee in Berlin (800 ha; shallow; eutrophic; 52°26′57″ N, 13°38′59″ E). Inflow into the ponds was about 1 ls^{-1} unfiltered lake water. Ponds were carefully cleaned before the experiment, and the bottom was covered with clean gravel (diameter 2–5 mm). Despite the absence of fish predators in the ponds, sources of predation risk in the pond environment were present through fish-eating birds regularly visiting the outdoor ponds and potentially through the presence of olfactory cues by predatory fish introduced into the ponds through the inflow of Müggelsee. Fish in the ponds were thus assumed to be permanently faced with some degree of latent predation risk, reflecting the generally mild latent risk-conditions present in commercial carp aquaculture ponds, i.e., the environment that the study animals have adapted to in the past.

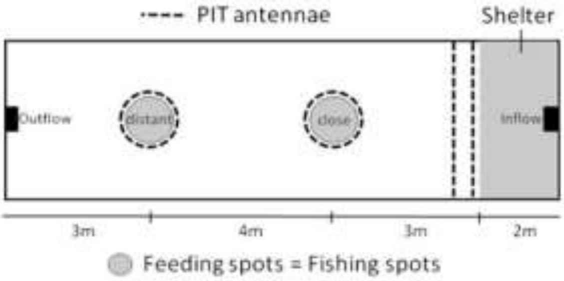

Fig. 1 Setup of the passive telemetry system used in the laboratory and ponds. We installed four antenna loops recognizing individual fish passages through the shelter entrance or when visiting the feeding spots. Two of the antenna loops were installed in front of the shelter to reveal the swimming direction of the fish from subsequent recordings. Another two antennae were used to cover the feeding spots. These antennae consisted of a circle and were placed at the bottom, providing detections of fish directly entering the circle. All data collected by the system were stored on a datalogger (Internal datalogger board, Texas Instruments, Dallas, TX, USA) and downloaded daily. Food was provided at the middle of the round feeding spots. When artificial predation risk was implemented, the baited hook was also placed in the middle of the feeding spot circle

The ponds contained a shelter structure (2 m×5 m) made out of black plastic material just above the water surface next to the water inlet and close and distant feeding spots (0.5 m diameter each) in different distances to the shelter (Fig. 1). All of these three structures were covered by PIT antennae (Fig. 1) so that we were able to quantify the individual number of visits at the feedings spots and the time spent sheltering as three measures of boldness. Because fish were allowed to acclimatize within the pond setup, the environment was not new to the fish, yet potentially risky, and behavioral measurements were thus considered indicative of boldness (Réale et al. 2007), but not indicative of exploratory behavior (Réale et al. 2007). The shelter structure was assumed to be perceived by the fish as the safest habitat, but lacking abundant feeding opportunities. Thus, foraging was only possible by taking the risk of leaving the shelter structure. All fish entering the feeding spots had to cross the large open pond area, comparable to variants of an open-field test (Budaev 1997). Open areas are more attractive to bold fish (Sneddon 2003), and we assumed bolder fish to show increased presence at the feeding spots. Fish were fed on the feeding spots on seven consecutive days to determine the foraging activity in the absence of any experimentally induced predation risk. This provided an initial measure of boldness. Feeding started 2 h before sunset until 2 h after sunset to control for potential impacts of daytime on boldness measurements. Feeding was conducted on a 60-min basis, alternating between the two feeding spots. Standard carp pellets (5 mm diameter, see previous discussion) were used as food, and the total daily food amount of pellets was 1% of the fish body wet mass at the time of stocking. In addition, for every single pellet, one sweet corn (5–7 mm diameter, Bonduelle, Reutlingen, Germany) was offered to also provide novel, yet preferred food for carp (Klefoth et al., unpublished data).

To test for potential behavioral changes of scaled and mirror carp in response to artificial predation risk, angling was conducted for another seven consecutive days. Angling was assumed to be perceived by the fish as a standardized, neutral, and mild form of artificially induced predation risk because learned hook avoidance as a consequence of hooking and subsequent live-release has been documented in carp (Beukema 1969; Raat 1985). Angling took place simultaneously to the daily feeding sessions and on the same spots. Sweet corn was used as bait, provided on a bold-rig as described by Rapp et al. (2008). This method ensured exclusive shallow hooking of the fish. The hook was connected to a 13-cm multifilament soft leader. The angling equipment consisted of a 3-kg monofilament line and a short fishing rod. Bites were indicated by an electronic bite indicator (Carp-Sounder Basic VR, Carp-sounder, Germany). After hooking, the fish was landed quickly using a small rubber net to prevent mucus abrasion (Barthel et al. 2003). Fish were then placed into a bucket filled with fresh water for unhooking and PIT identification (Pocket reader, Allflex, Dallas, TX, USA). Afterwards, fish were immediately released in the middle between the two feeding spots. Release of the fish was always conducted within 30 s, and no mortality occurred. The whole experimental procedure within the pond environment lasted 14 consecutive days (7 days of feeding without angling-induced risk, followed by 7 days of feeding under angling-induced risk).

The environmental conditions in the ponds were documented using temperature loggers (TidbiT datalogger, Onset, Bourne, MA, USA) and using data from a weather station located 500 m away from the Müggelsee, providing data on an hourly basis for wind speed (m s^{-1}), global radiation (W m^{-2}), light intensity (μmol m^{2}–^{1}) at 0.75-m water depth, air pressure (mbar), humidity (%), and air temperature (°C). Mean water temperature ± SD in the ponds over the study period was 19.0±0.5°C (range 17.0–20.2°C).

Behavioral experiments under laboratory conditions

We conducted an additional laboratory-based experiment under controlled environmental conditions to test for the consistency of boldness differences among the two carp genotypes under fully controlled conditions. This experiment resembled the setup established in the ponds and used a new set of study animals (see previous discussion). Experiments were conducted in a large laboratory tank (10 m×4 m×1 m; $L\times W\times H$) of comparable size to the ponds. The tank was connected to a circulating water system and a biological filter. Water inflow was 2 ls^{-1}, and water temperature ± SD was constant at 22±1°C. To investigate boldness parameters in the laboratory, a total of 40 similar-sized fish (20 scaled carp and 20 mirror carp, mean TL ± SD 225±20.0 and 229±16.0 mm, respectively, *T*-Test, $t=-0.80$, $P=0.441$) were stocked into the tank and allowed to acclimatize for 2 days before behavioral observations started. Behavioral experiments followed the same protocol described for the pond experiment and assessed the same behavioral variables of boldness, with the exception that the intervals of changing the feeding spots within the daily 4-h feeding periods were 15 min instead of 60 min for logistical reasons. After the 7th day of angling within the laboratory environment, experimental carp were replaced by a new set of 40 fish (20 scaled carp and 20 mirror carp, mean TL ± SD 224±17.0 and 229±21.0 mm, respectively, *T*-Test, $t=-0.89$, $P=0.377$), and the experiment was replicated.

Data recording and statistics

From the raw PIT data, we calculated the boldness parameters "time spent sheltering" (min h^{-1}) and "number of visits at the feeding spot" (# h^{-1}), with the latter separately for the close and distant feeding spots. We defined a fish to be sheltering after it had passed the PIT antennae in front of the shelter (Fig. 1) in a direction from the outside to the inside of the shelter. Sheltering activities ended when the fish passed the antennae in the opposite direction. If a fish was not detected at both antennae, sheltering ended when the fish was observed to be elsewhere than the shelter. Visits at the feeding spots were defined by single observations of individual fish. To prevent overestimation of visits by multiple detections within a short time frame in which the fish did not leave the feeding spot, an interval of 30 s was applied before a new visit was counted. Pretest experiments showed that the maximum time fish spent within the circle antennae was always less than 30 s (Klefoth et al., unpublished data), justifying our assumption. Because the number of visits at the close and distant feeding spots was highly correlated (Spearman's rho $=0.88$, $p<0.001$, in the pond environment, and Spearman's rho $=0.78$, $p<0.001$, within the laboratory environment), the mean number of visits at the close and distant feeding spots per individual and per unit time was used for subsequent analyses, resulting in a single variable describing the number of visits at the feeding spots. No such strong correlations were found between the number of visits at the feeding spots and the time spent sheltering (all Spearman's rho <0.5). Therefore, the time spent sheltering was used as an additional boldness-related parameter.

Functionality of the PIT system was tested in a first trial prior to conducting the pond and laboratory studies with different fish within the same general setup. This was done because stationary PIT systems are known to be limited in their ability to detect multiple individuals at the same time (Zydlewski et al. 2001). We assessed the behavioral measures—"time spent under shelter" and "number of visits at the feeding spots" estimated from the raw PIT data—and tested for correlations with visual observations of the same parameters. Results using Spearman correlations between observed and calculated data for the time spent under shelter for 10-min periods (Spearman's rho $=0.474$, $P=0.030$, $N=18$) and the number of visits at the feeding spots during 10-min periods (Spearman's rho $=0.575$, $P<0.001$, $N=36$) confirmed a high functional capability of the PIT system to remotely measure boldness-related traits of carp (Klefoth et al., unpublished data). All calculations of boldness parameters were conducted for feeding and non-feeding periods for every fish on a daily basis (two data points per fish and day). To standardize for differences in the duration of feeding periods (4 h) and non-feeding periods (rest of the day), mean values per hour were calculated.

We used Generalized Linear Mixed Models (GLMM) to explain sheltering activities (min h^{-1}) and mean visits at the feeding spots (# h^{-1}) in the pond environment and the laboratory. Fish ID nested within pond or tank replicate was added as random factor to account for repeated measures and the nested structure of the experiments. We used the dataset to test for differences in the behavioral response of scaled and mirror carp (Genotype) to food supply without risk (Feeding) and the period when artificial predation risk was implemented while feeding (Risk). Individual TL was added as covariate to all models because no differences between within and between subject effects were identified when centering TL within ponds as outlined by van de Pol and Wright (2009). Previous capture and release events (Capture) were considered in the model as well to control for potential impacts on subsequent behavior of the fish (Klefoth et al. 2008; 2011). All possible two-way and three-way interactions with Genotype, Feeding, and Risk were added to the models. In all cases, data were overdispersed, and a quasi-Poisson error distribution was found to be the best fit to the data. We used the software package R and the Penalized Quasi-Likelihood method (function glmmPQL) in library MASS (R Development Core Team 2009). Variances explained by the models were calculated using the "predict method for glmmPQL," also provided in library MASS. Predicted values were regressed against observed values using linear regression.

To account for uncontrolled environmental conditions in the ponds, we conducted a PCA with varimax rotation on all environmental data collected, generating two components [variable and factor loading, respectively: global radiation (0.971), light intensity (0.870), humidity (−0.885), air temperature (0.984) (PC1, explained variance: 57.1%, eigenvalue: 3.4); wind speed (−0.545), air pressure (0.802) (PC2, explained variance: 17.3%, eigenvalue: 1.1)], together explaining 74.4% of the total variance. In initial models, the estimated variance components of factor scores for PC1, PC2, and water temperature were generally low (< 6%). Therefore, environmental parameters were removed from further analyses.

Results

Comparisons of boldness-related behaviors between scaled and mirror carp strongly differed between the two ecological contexts studied (Figs. 2 and 3). In the pond environment, the main effect of Genotype was found to consistently and significantly explain visits at feeding spots independent of ecological context. Importantly, no significant interactions between Genotype and the environmental factors Feeding or angling-induced Risk on feeding spots were present (Table 1; Fig. 2), underscoring the robustness of the genotypic differences in foraging behavior in the pond environment, even in the absence of natural fish predators. In line with expectations, the more domesticated mirror carp were found twice as often on the feeding spots compared to their less domesticated scaled conspecifics (Table 1; Fig. 2). In addition to Genotype, the fixed effects Feeding and Risk were also found to significantly affect foraging behavior in the pond environment. Accordingly, in periods where food was supplied on feeding spots, the number of visits by both genotypes was generally higher, and also under these conditions, mirror carp visited the feeding spots more frequently than scaled carp. For example, the mean number of visits ± SD at feeding spots while feeding and before implementation of angling-induced risk of mirror carp and scaled carp was 3.2±3.0 and 1.6±1.8 visits h^{-1}, respectively (Table 1; Fig. 2). Interestingly, during periods when angling-induced risk was present in addition to food on the feeding spots, fish in the ponds were more often found on the feeding spots compared to periods without risk (mean number of visits ± SD at feeding spots while feeding and after

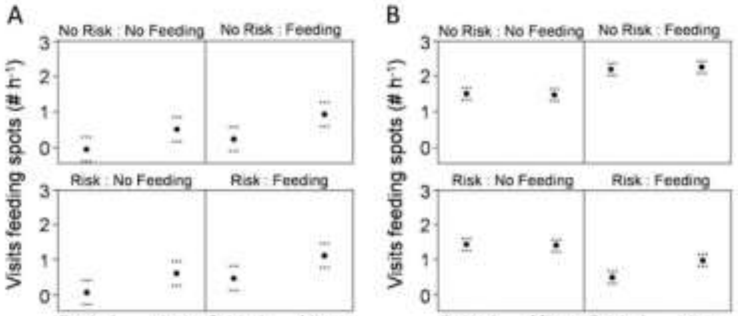

Fig. 2 Behavior of scaled and mirror carp in response to feeding and non-feeding times with and without implementation of risk through angling **A** in ponds and **B** in the laboratory. The figure shows the least squares means and the 95% confidence intervals of the mean number of visits at feeding spots (# h^{-1}). Bold p-values indicate significant effects

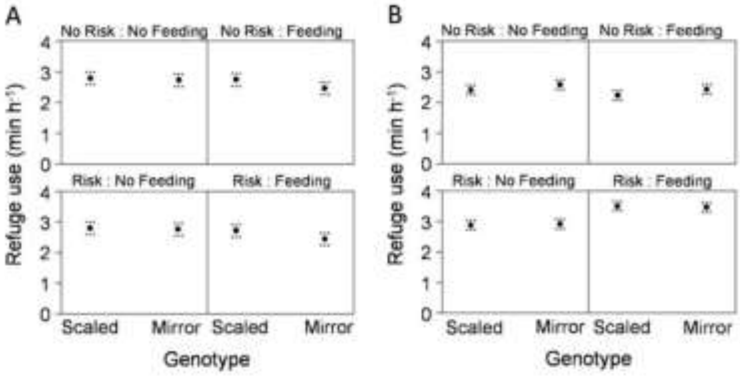

Fig. 3 Behavior of scaled and mirror carp in response to feeding and non-feeding times with and without implementation of risk through angling **A** in ponds and **B** in the laboratory. The figure shows the least squares means and the 95% confidence intervals of the time spent sheltering (min h^{-1}). Bold p-values indicate significant effects

implementation of angling-induced risk for mirror carp and scaled carp 4.0±3.5 and 2.1±2.4 visits h^{-1}, respectively, Table 1; Fig. 2). In addition to the aforementioned main effects, in the ponds, we also found the size of the carp to be positively related to the number of visits at the feeding spots (Table 1).

A different picture was evident in the laboratory, where the main effect Genotype was significantly interacting with the other main effects Feeding and Risk to explain the number of visits at feeding spots by scaled and mirror carp in a more complex manner (Table 1; Fig. 2). Therefore, the revealing of boldness differences of the two genotypes in terms of foraging was driven by the environment, and thus, it is context dependent. The provision of food in the absence of risk on feeding spots generally increased the number of food patch visits (Fig. 2). However, in contrast to the pattern in the pond, the onset of angling on previously risk-free feeding spots reduced the frequency of feeding spot visits by both genotypes in absolute terms when food was supplied in the laboratory. In fact, the mean number of visits declined by approximately 83% to 1.5±2.7 visits h^{-1} for scaled carp and by 73% to 2.6±3.5 visits h^{-1} for mirror carp after implementation of angling-induced risk, in turn reaching levels that were present in the pond already at the onset of the

Table 1 Nested Generalized Linear Mixed Models (GLMM) to predict the effects of Genotype, Feeding, Risk (induced by angling), and previous Capture and all relevant two-way and three-way interactions on the mean number of visits at the feeding spots (# h^{-1}) in the pond environment (*left*) and the laboratory (*right*). All models included individual fish nested within pond or laboratory replicate as a random effect. Total length was considered as covariate

Fixed effects	Estimate ± SE	DF	*T*	*P*	R^2	Fixed effects	Estimate ± SD	DF	*T*	*P*	R^2
Pond feeding spots					0.43	Lab feeding spots					0.48
Intercept	−4.21±1.84	2,605	−2.3			Intercept	1.23±0.36	2,011	3.5		
Genotype[a]	0.59±0.17	112	3.5	**<0.001**		Genotype[a]	−0.06±0.07	74	−0.8	0.403	
Feeding[b]	0.31±0.06	2,605	4.9	**0.001**		Feeding[b]	0.69±0.04	2,011	19.7	**<0.001**	
Risk[c]	0.16±0.07	2,605	2.5	**0.013**		Risk[c]	−0.06±0.04	2,011	−1.4	0.158	
Capture[d]	−0.20±0.24	2,605	−0.8	0.405		Capture[d]	−0.23±0.09	2,011	−2.5	**0.012**	
Length	0.02±0.01	112	2.2	**0.028**		Length	0.00±0.00	74	0.8	0.438	
Genotype × Capture	−0.13±0.27	2,605	−0.5	0.629		Genotype × Capture	0.08±0.11	2,011	0.7	0.502	
Genotype × Feeding	0.13±0.08	2,605	1.6	0.112		Genotype × Feeding	0.12±0.05	2,011	2.3	**0.023**	
Genotype × Risk	−0.03±0.08	2,605	−0.4	0.723		Genotype × Risk	0.03±0.06	2,011	0.4	0.683	
Feeding × Risk	0.09±0.08	2,605	1.0	0.302		Feeding × Risk	−1.63±0.07	2,011	−23.9	**<0.001**	
Genotype × Feeding × Risk	−0.01±0.11	2,605	−0.1	0.916		Genotype × Feeding × Risk	0.40±0.09	2,011	4.4	**<0.001**	

[a] Reference is scaled carp

[b] Reference is non-feeding times

[c] Reference is period without angling-induced risk

[d] Reference is not being captured previously

experiment in the absence of predation risk and subsequently during food supply (Fig. 2). In contrast to the situation in the pond, in the laboratory, the number of visits at the feeding spots was almost equal for both genotypes before angling started, both within and outside feeding times (Fig. 2), and it remained so after angling started in periods lacking food supply. However, the use of feeding spots diverged between the genotypes once fishing started in periods when food was supplied, and the visits were then found to be, on average, 67% higher for mirror carp relative to scaled carp. These combined results indicate that there were indeed genetically based differences in the propensity to forage among scaled and mirror carp, but the revealing of these differences in the laboratory was strongly context dependent and only occurred under conditions of food provision and the presence of predation risk. In addition to Genotype, Feeding, and Risk, previous capture events also affected the future number of visits at the feeding spots by reducing their frequency in the laboratory (Table 1).

In terms of sheltering activities, a few significant effects were found to predict refuge use within the pond environment, and in contrast to the foraging behavior, no significant context-independent main effect of Genotype was present. However, in line with expectations, mirror carp spent significantly less time under shelter compared to their scaled conspecifics when food was supplied, as revealed by a significant Genotype × Feeding interaction (Table 2; Fig. 3), and this pattern was unaffected by the presence or absence of angling-induced risk (non-significant Genotype × Feeding × Risk interaction, Table 2; Fig. 3). This result indicates that mirror carp behaved generally bolder in terms of refuge use during food supply within the ponds than scaled carp, irrespective of the existence of more explicit angling-induced risk-stimuli.

The findings on sheltering were less conclusive in the laboratory. Both genotypes similarly increased sheltering activities when food was supplied during the periods of angling-induced risk compared to feeding in the absence of angling (significant Feeding × Risk interaction, Table 2; Fig. 3). In terms of differences among genotypes, refuge use by scaled carp increased in a somewhat more pronounced fashion in response to the onset of angling-induced risk relative to the similarly expressed average increase in sheltering shown by their mirror carp conspecifics, resulting in a significant Genotype × Risk interaction. However, absolute and relative differences among genotypes in terms of refuge use in the laboratory were small and statistically independent of feeding (non-significant Genotype × Feeding × Risk interaction, Table 2; Fig. 3), although descriptively they were most clearly expressed in the absence of feeding. These results overall reveal that the generally small differences in sheltering activities between scaled and mirror carp

Table 2 Nested Generalized Linear Mixed Models to predict the effects of Genotype, Feeding, Risk (induced by angling), and previous Capture and all relevant two-way and three-way interactions on the time spent sheltering (min h^{-1}) in the pond environment (*left*) and the laboratory (*right*). All models included individual fish nested within pond or laboratory replicate as a random effect. Total length was considered as covariate

Fixed effects	Estimate ± SD	DF	*T*	*P*	R^2	Fixed effects	Estimate ± SD	DF	T	P	R^2
Pond shelter structure					0.19	Lab shelter structure					0.38
Intercept	2.64±0.88	2,605	3.0			Intercept	2.46±0.27	2,011	9.1		
Genotype[a]	−0.07±0.09	112	−0.8	0.445		Genotype[a]	0.18±0.06	74	2.8	**0.006**	
Feeding[b]	−0.05±0.05	2,605	1.1	0.281		Feeding[b]	−0.18±0.05	2,011	−3.3	**<0.001**	
Risk[c]	−0.01±0.05	2,605	−0.2	0.847		Risk[c]	0.48±0.05	2,011	10.2	**<0.001**	
Capture[d]	0.17±0.2	2,605	0.8	0.402		Capture[d]	−0.04±0.07	2,011	−0.6	0.568	
Length	0.00±0.0	112	0.2	0.868		Length	−0.01±0.00	74	−0.2	0.841	
Genotype × Capture	0.01±0.24	2,605	0.0	0.979		Genotype × Capture	0.06±0.08	2,011	0.7	0.472	
Genotype × Feeding	−0.21±0.07	2,605	−2.9	**0.004**		Genotype × Feeding	0.02±0.07	2,011	0.3	0.768	
Genotype × Risk	0.04±0.07	2,605	0.6	0.581		Genotype × Risk	−0.14±0.07	2,011	−2.1	**0.035**	
Feeding × Risk	−0.01±0.07	2,605	−0.2	0.836		Feeding × Risk	0.79±0.07	2,011	12.1	**<0.001**	
Genotype × Feeding × Risk	−0.04±0.10	2,605	−0.4	0.684		Genotype × Feeding × Risk	−0.10±0.09	2,011	−1.1	0.289	

[a] Reference is scaled carp

[b] Reference is non-feeding times

[c] Reference is period without angling-induced risk

[d] Reference is not being captured previously

within the laboratory mainly depended on the implementation of angling-induced predation risk and that there were no differences in behavioral expressions of scaled and mirror carp regarding their basal level of refuge seeking in the laboratory in the absence of risk.

Discussion

Our study revealed the expected difference in boldness-related behaviors among two genotypes of differently domesticated carp in terms of a higher average boldness of the more strongly domesticated mirror carp relative to scaled carp, particularly in relation to feeding behavior in the presence of predation risk by angling on feeding spots and to some degree also in relation to refuge use as a second dimension of boldness. However, this conclusion was only unambiguous when the two genotypes of carp were tested under pond conditions. In the laboratory, the addition of artificial predation risk to the test trials was needed to more clearly reveal boldness differences among the two carp populations, and again, this was mainly the case for feeding-related behaviors and less pronounced for refuge use. The importance of tests conducted in tank versus pond environments and the impact of predation risk-stimuli on study outcomes in the laboratory indicated the existence of genotype × environment interactions as it relates to boldness expressed by genetically distinct populations of carp. Simply applying a variant of a classical open-field test, which is often assumed to reliably measure boldness in fish (e.g., Budaev et al. 1999; Brown et al. 2007), in a large laboratory tank would thus have provided inconclusive or even misleading results in terms of adapted boldness differences among the two carp strains. Most importantly, one would probably not have concluded a genetic basis of boldness differences among the two carp genotypes using a laboratory experiment alone, without addition of artificial predation risk to the behavioral assay. This is because an open-field test without additional risk-stimuli under laboratory conditions may not separate the effects of the genotype from potentially important genotype × environment interactions. Furthermore, by only applying open-field tests without behavioral observations under risk, one may not be able to distinguish between adapted behavioral responses towards predation risk and genetic adaptations of basal boldness as, for example, measured by exploration of a non-novel, yet potentially risky open area between the refuge and the feeding spots, and our study underscores that carp genotypes may express these traits differently depending on ecological contexts. The general similarity of our tank and pond experimental setups (e.g., both were lacking fish predators) gave rise to important differences in study findings as it relates to boldness differences of carp. We raise to mind to not prematurely discard the possibility for genetic adaptation of fish populations in terms of boldness, even if this pattern is not immediately revealed in an open-field laboratory study that controls all other potentially "confounding" environmental factors. Potentially, one then needs to implement some form of predation risk to reveal genetic variance in non-basal dimensions of boldness.

Our study showed large effects of the ecological context (pond vs. laboratory environment) and ecological factors (existence of predation risk-stimuli within the laboratory environment) on the expression of boldness-related traits of two genotypes of carp that were expected to generally differ in basal boldness due to genetic adaptation to low-risk aquaculture conditions. Because consistent and context-independent behavioral differences between scaled and mirror carp were only found in the pond environment, our study underscores earlier recommendations on the design of comparative studies in fish if these are aimed at revealing the genetic adaptation of behaviors to key local ecological factors. Either such studies are to be conducted using common-garden reared offspring in the laboratory, which allows the removal of confounding environmental variation and the "clean" testing of individual environmental factors (e.g., risk of predation or food supply). However, as our study showed, experimenters may not reveal the true picture of boldness adaptation if the correct environmental stimuli are missing. An alternative perspective may be, in light of the lack of clear boldness differences among our carp strains, that selection has not been strong enough to change basal boldness expected to be expressed in an open-field test in the absence of explicit predation risk (Brown et al. 2007). However, we contend that studies on genetic adaptation of behavior should also be conducted under less controlled conditions by exposing test populations to a range of natural environmental factors supposed to be involved in their evolution. If technically feasible, such studies may be conducted within the original evolutionary environment using reciprocal transplant approaches (Kawecki and Ebert 2004; Walling et al. 2004) or in ecological conditions reasonably close to those of the original evolutionary environment. We contend that the experimental ponds that we used in our carp studies represented a reasonable approximation of the original evolutionary environment, and maybe not surprisingly, the differences in boldness among mirror and scaled carp were robust and clear in this pond environment, even in the absence of any additional predation risk (e.g., also in basal levels of boldness).

Results of our study suggest that when comparative population studies on the genetic variance of boldness of fish are conducted in the laboratory, careful choice of the ecological context and the appropriate predation risk-stimuli may be needed to reveal robust results. Ideally, the laboratory may also mimic the original environmental conditions as close as possible (Kawecki and Ebert 2004), although researchers should keep in mind that with increasing complexity of the experimental setup, uncontrolled environmental effects or complex genotype × environment interactions might complicate study results. Thus, standardized experimental protocols and setups allowing isolation of the behavioral responses of interest should generally be favored over trying to mimic nature in laboratory environments. Thereby, the benefits of laboratory trials (designed to isolate cause and effects) may ideally be combined with the strength of more natural environments (designed to study individual responses to a suite of correlated or uncorrelated natural factors) using common-garden reared individuals if studies are indented to reveal patterns of local adaptation. We urge, however, to be careful about implicating about the lack of genetic adaptation if laboratory results do not reveal the expected patterns. One might have missed to include the appropriate test stimulus or generally measured the wrong trait that has not been under divergent selection in nature.

Our findings showed that consistency of boldness-related traits in fish can be impacted by the presence or absence of stressful situations like those induced by predation risk. Earlier studies have shown that randomness of behavioral expressions tends to be predominantly pronounced in non-threatening situations (Alados et al. 1996; Budaev et al. 1999), and in our study, the lack of boldness differences among carp strains in the absence of angling in the laboratory shows that the large open field was likely not perceived as threatening by the fish, presumably facilitated by rapid learning and habituation as no other predators were present in the fully controlled laboratory tank. Therefore, we contend that one should attempt to measure several dimensions of boldness in laboratory studies to avoid inappropriate conclusions based on a restricted set of measures that may capture different dimensions of the composite trait boldness. Thus, the internal validity of boldness-related measures under laboratory conditions should be highest by incorporating several different measures of boldness, including observations under predation risk (Toms et al. 2010), thereby considering potential interaction effects of the genotype and the environment (Gerlai and Csányi 1990) and also distinguishing between adaptation of boldness-related traits on the basal level (as, for example, revealed in open-field tests) and in relation to more explicit risk of predation (as, for example, revealed in our experiment by using angling on previously safe feeding spots).

In the pond environment, we revealed mirror carp to be generally and consistently bolder than scaled carp. Though no natural predatory events were observed during the study period, the presence of fish-eating birds in the pond area was observed repeatedly—a factor that is known to influence the foraging behavior and sheltering activity of fish (Allouche and Gaudin 2001). Sources of latent predation risk in the pond environment might have also been based on olfactory cues by predatory fish despite the absence of fish predators in the experimental ponds. This is because all ponds were provided with water from a large natural lake, potentially containing chemical cues from predatory fish such as pike. Aquatic animals evolve sensitive receptors for detecting these cues for the assessment of predation risk (Wisenden 2000), and prey can smell chemical cues of their predators, even if they have never encountered the predator (Chivers and Smith 1998; Kats and Dill 1998). We thus assume that the existence of latent predation risk was responsible for consistent differences in boldness among our carp populations, also in the absence of artificial predation risk by angling. This conclusion was reinforced by our laboratory findings, where boldness differences among carp populations were only evident when risk-stimuli were introduced into the experiment and where the number of visits at the feeding spots and the time spent sheltering significantly changed after implementation of risk. Furthermore, in the laboratory, the number of visits of carp at the feeding spots reached comparable levels to those within the pond environment, but only when predation risk in the form of angling was introduced. This suggests that the less frequent use of feeding spots in the pond compared to the laboratory in the absence of angling may reflect the "standard" behavior of carp when latent predation risk is present.

Generally, our findings, particularly those from the ponds, were in agreement with a wide range of other studies comparing the behavior of common-garden reared fish from high-and low-predation sites in the laboratory (e.g., Huntingford and Wright 1992; Magurran et al. 1992; Bell and Stamps 2004; Ghalambor et al. 2004). In line with our results, all of these studies showed that fish adapted to low-risk conditions were, on average, bolder than their high-risk conspecifics when faced with artificially implemented or natural risk-stimuli. Opposing findings in the literature (Brown et al. 2007) might be related to locally different selection pressures or have a methodological cause by only measuring boldness-related traits on the basal level and omission of tests with more explicit risk of predation. Moreover, fish tend to exhibit high plasticity in terms of expression of behavioral phenotypes (Dingemanse et al. 2010; Stamps and Groothuis 2010) such that testing of wild-captured fish with a life-time experience in a high-predation environment may exhibit greater boldness compared to low-predation conspecifics. The most robust information about the genetic basis of behavioral traits can be expected by using common-garden reared fish (Kawecki and Ebert 2004), and thus, we consider our findings on the differences in boldness among our two carp strains to have a genetic origin.

In addition to the importance of common-garden protocols, our study also highlights the importance of considering potential effects of genotype × environment interactions in laboratory protocols designed for among-population comparisons of boldness in fish. However, in much of the current fish behavioral literature, the importance of standardized risk-stimuli in assessments of boldness seems to be underappreciated, and various researchers employ different predation-stimuli in their boldness tests (Toms et al. 2010; Conrad et al. 2011), potentially influencing study outcomes. The absence of a standardized experimental protocol for boldness-related measurements also constrains the comparability of studies and may affect the reliability of study findings in potentially important ways. Brown et al. (2007) argued that perception of predation threats might differ between fish adapted to high-risk and low-risk conditions. This makes it difficult to distinguish if observed behavioral differences in the presence of predators are based on adapted differences in boldness or adapted differences in threat recognition, in turn motivating the use of open-field tests as a clean measure of basal boldness of fish (Brown et al. 2007). However, as our laboratory experiment has shown, genetic variance in basal boldness in an open-field test may only emerge in the presence of latent predation risk. Thus, the expression of basal boldness might be a function of the perception of some level of predation risk, and open-field tests might not necessarily offer this degree of functionally important level of risk, at least not within the laboratory in common carp. Thus, to generate robust findings in studies on adaptation of populations, we recommend inclusion of different setups, including different behavioral measurements with and without explicit risk to more fully elucidate the genetic adaptation of the behavioral repertoire of fish populations to predation risk in the wild. In this way, the effects of genotype, environments, and genotype × environment interactions can be better understood, leading to an improved understanding of the adaptive divergence of the focal trait.

We choose to implement angling on feeding spots as an experimental inclusion of predatory threat to avoid using real predators or predator models, thereby circumventing the issue of differential threat recognition evolution to natural predators (Brown et al. 2007). We assumed angling to constitute a neutral risk-stimulus as hook avoidance learning was previously documented in carp angled in pond environments (Beukema 1969; Raat 1985). The fact that we could observe behavioral alterations towards angling-induced risk only within the laboratory environment (as indicated by a reduced frequency of visiting feeding spots) suggested two implications. First, angling was perceived as threatening in the laboratory, leading to a reduced usage of feeding spots, and the level of threat for carp in the pond environment was not strong enough to further reduce a basal level of visits at feeding spots. The very similar level of visits on feeding spots per individual and hour was also reached in the laboratory after angling started, collectively indicating that while angling was surely perceived as a threat, feeding spots did not completely lose their attraction to our study fish.

We found that the experience of previous capture significantly reduced the number of visits at the feeding spots in the laboratory. This can be explained by learning effects as described previously (Beukema 1969), which may have been more pronounced in the cognitively simpler tank environment (Girvan and Braithwaite 1998) and be facilitated by greater water clarity that may have helped carp to identify angling gear and avoid being hooked. However, capture was also a covariate in the pond model so that any capture-related effects on boldness-related behaviors were statistically controlled, and the overall study findings were robust.

There were few differences in the experimental setup between the tank and pond environments, and this might have influenced the study findings (e.g., different temperature, environmental exposure, and water clarity). Moreover, fish used in the laboratory approach were slightly larger in size than those used within the ponds. However, size of the fish did not differ between the two genotypes in any of the ponds or tank replicates. In addition, we used total length of individual fish as a covariate in our statistical models, and thus, we are certain that any behavioral differences between scaled and mirror carp on the population level were not caused by the size of study animals. However, we found a significant and positive relationship between the size of the fish and the number of visits at the feeding spots within the pond environment. It has been repeatedly shown that the basal levels of boldness in fish are independent of the size of the fish (Sundström et al. 2004; Brown et al. 2005), but instead larger size attained by a given fish can be a consequence of bold behavior (Johnsson 1993). Because our fish were raised within a natural pond with regular food supply prior to experimentation, among-individual differences in size might correlate with boldness and related higher feed intake rates, potentially explaining why larger fish were more often found on the feeding spots within the pond environment.

In conclusion, our study revealed interactions between the genotype of carp and the ecological environment in which boldness was measured. The genetic basis of boldness differences among the two populations of carp was unambiguous in the more natural pond environment, even in the absence of fish predators and angling-induced predation risk. Similar behavioral differences between our two genotypes of carp were also found in the laboratory when tested under risk of predation, highlighting the potential for adapted behavioral responses towards predation risk rather than basal boldness expressions per se. Due to the common-garden approach, our study provides evidence about genetic adaptation of boldness in carp (particularly in response to predation risk). From a methodological perspective, our study underscores the suggestions by Kawecki and Ebert (2004) that robust local adaptation studies should ideally be conducted under natural conditions or in laboratory conditions involving a range of experimental stimuli. Reciprocal transplant studies in the wild are one possible way for the future that can also take advantage of modern tracking technologies like PIT systems, as applied in our experimental study. This may also help in eliminating the potential for observer bias effects through remote observation of individual behavioral patterns. In this way, evolution of behavioral traits in response to different predator regimes or other ecological factors can more realistically be studied without the potential for experimentally induced complications through genotype × environment interactions that may lead to erogenous conclusions. Alternatively, boldness-related measures under laboratory conditions should incorporate various boldness measures, including observations with and without explicit predation risk, thereby considering potential interaction effects of the genotype and the environment and also distinguishing between adaptation of boldness-related traits on the basal level and in response to explicit predation risk.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical note Animal handling associated with this study was approved through an animal care permit (*No G 0178/09*) granted by the State Office of Health and Social Affairs in Berlin in accordance with the German Animal Protection Act.

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Paper III

III

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Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*)

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ABSTRACT

Non-lethal assessments of proximate body composition of fish can help unravelling the physiological and condition-dependent mechanisms of individual responses to ecological challenges. Common non-lethal methods designed to index nutrient composition in fish include the relative condition factor (K_n), bioelectric impedance-based assessments of body composition (BIA), and microwave-based "fat" meters (FM). Previous studies have revealed mixed findings as to the reliability of each of these. We compared the performance of K_n, BIA and FM at different temperatures to predict energetic status of the whole bodies of live eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*) and the dorsal white muscle of carp. Homogenized fish flesh was used for calibration. Relative dry mass was strongly correlated with relative fat content (R^2 up to 96.7%) and energy density (R^2 up to 99.1%). Thus, calibrations were only conducted for relative dry mass as an index of energetic status of a fish. FM readings were found to predict relative dry mass of whole body in eel ($R^2 = 0.707$) and carp ($R^2 = 0.676$), and dorsal white muscle of carp ($R^2 = 0.814$) well. By contrast, BIA measurements and K_n were much less suited to identify variation in relative dry mass. BIA-based models were also temperature-dependent. As a result, a regression model calibrated at 10 °C and applied to BIA measurements at 20 °C was found to underestimate energetic status of a fish. By contrast, no effects of temperature on FM calibration results were found. Based on our study, the FM approach is the most suitable method to non-lethally estimate energetic status in both, carp and eel, whereas BIA is of limited use for energetic measurements in the same species, in contrast to other reports in the literature.

1. Introduction

Proximate body composition of fish, usually measured as the relative amount of moisture, lipid, protein, and ash within fish flesh, is influenced by a range of exogenous and endogenous factors (Shearer, 1994). Macronutrient composition in fish flesh constitutes an integrative endpoint of complex ecological processes that involve catabolism and anabolism and is also a key determinant of behaviour, maturation and survival, e.g., over winter (Ursin, 1967; Gardiner and Geddes, 1980; Post and Parkinson, 2001; Biro et al., 2005). Nutrient content and the composition of nutrients in fish flesh thus provides important insights into the physiological and energetic status of fish, which in turn can help predicting an individual's condition for wintering (Schreckenbach et al., 2001) or its propensity to engage in migration (Larsson et al., 1990) or spawning (Ludsin and DeVries, 1997). The proximate body composition of fish is usually measured in tissue samples taken from sacrificed fish (e.g., Hendry et al., 1999; Mathes et al., 2010). Such studies can only offer a snap-shot picture at the population-level, because individual fish cannot be tracked over time. Non-lethal assessment techniques of body composition in fish were developed to offer an alternative that allows for repeated measures on individual fish to study fitness in the wild or in aquaculture conditions.

A range of non-lethal methods have been developed. The earliest were length–weight-regression-based condition indices such as Fulton's condition factor (Ricker, 1975) or the relative condition factor (K_n), which relates an individual's actual weight to a standard average weight in the studied fish population (Le Cren, 1951). However, length–weight relationships are not without problems when

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used to index condition, because relationships change through ontogeny and seasonally, e.g. during spawning periods (Vøllestad and Jonsson, 1986; Froese, 2006). Thus, length–weight-based condition indices should only be used after careful examination of their underlying assumptions (Bolger and Connolly, 1989; Cone, 1989).

A further opportunity is to analyze the ratio of dry mass to wet mass of an individual (Hartman and Brandt, 1995). Due to strongly inverse relations of water and lipid contents in fish flesh (Schreckenbach et al., 2001) a higher dry mass should correlate positively with energetic density and hence condition (Caulton and Bursell, 1977). Indeed, dry mass has been found to constitute a useful surrogate of energetic status of fish (Shearer, 1994). However, analysis of dry mass still requires obtaining a flesh sample of the fish, either by sacrificing the fish or by muscle biopsy as a non-lethal alternative approach (Hendry et al., 2001).

The latest technical developments for estimating proximate body composition and/or energetic status of fish were based on the inverse correlation of lipid content and water content. In these applications water content in fish flesh is measured using electric currents [bioelectric impedance analysis, BIA, Cox and Hartman (2005)] or microwaves in handheld devices [fat meters, FM, Crossin and Hinch (2005)]. Calibration studies using BIA devices regressed various nutrients in fish flesh against BIA readings, in particular total body water, body fat, body fat-free mass, protein, and ash across a range of species (brook trout *Salvelinus fontinalis* Cox and Hartman, 2005; Rasmussen et al., 2012; steelhead *Oncorhynchus* mykiss Hanson et al., 2010; yellow perch *Perca flavescens*, walleye *Sander vitreus*, lake whitefish *Coregonus clupeaformis* Pothoven et al., 2008; channel catfish *Ictalurus punctatus* Bosworth and Wolters, 2001). It is worth noting that BIA cannot measure any of these variables directly (Schoeller, 2000). In fact, BIA measures the resistance and its inverse, reactance, of an animal's body to an electrical current where resistance is dependent on the quantity (and not the properties) of intracellular and extracellular water. Thus, the quantity of water within fish flesh mainly influences the degree of resistance (Schoeller, 2000). Because body water is inversely related to body fat content as mentioned before (Craig, 1977; Schreckenbach et al., 2001) and hence water relates inversely to energy density (Chellappa et al., 1995), BIA measures have been found to correlate well with a range of body composition metrics in fish (e.g., Bosworth and Wolters, 2001; Pothoven et al., 2008; Hanson et al., 2010; Rasmussen et al., 2012).

FM devices are an alternative to BIA, and they are based on a sensor for microwave moisture measurements (Kent, 1992). The sensor is directly placed on the tissue of interest (e.g., fish muscle). Because materials with polar constituents like water can be described by complex dielectric permittivity where the material is able to store energy (Kent, 1992), the loss of energy in the sensor can be used to predict the water content of the material (Kent, 1992). Thus, similar to BIA, FM is supposed to measure the water content of the tissue of interest. FM readings have been regressed on species-specific nutrient composition values for calibration purposes of the device (Pacific salmon *Oncorhynchus* spp. Crossin and Hinch, 2005; North Sea herring *Clupea harengus* Davidson and Marshall, 2010). Using such calibration results, the commercially available FM device displays the result of the regression using a species-specific regression of FM readings and relative lipid content, and not what the device actually measured (i.e., energy loss). FM has been applied to study lipid levels in live fish (Crossin et al., 2008), dead fish (Quillet et al., 2005), and fillets (van Sang et al., 2009). Because FM assesses the water content of this tissue, regressions on dry mass in fish flesh should generate the most robust results, similar to BIA. All other predictions of BIA or FM outputs with body constituents such as protein, fat or ash, are likely to be more spurious and variable across species and ecological contexts.

Previous calibration studies that developed BIA reported correlations of device outputs (e.g., impedance measurements in BIA) with total (e.g., absolute g per individual) rather than relative nutrient levels [e.g., g per g fish flesh; Cox and Hartman (2005)]. However, the total mass of a proximate component should be strongly related to the size of the fish (Caulton and Bursell, 1977; Weatherley and Gill, 1983) and is therefore less suitable to discern inter-individual differences in relative body composition levels of fish that are of similar size. Ecologically it is often the relative differences among individuals that are of interest to the researcher (Beamish and Mahnken, 2001) and thus, it is important to calibrate BIA and FM devices also to relative measures of body composition.

So far, the effect of temperature on calibration quality of BIA in fish has only been considered in a single study (Hartman et al., 2011). However, the temperature dependency of impedance (BIA) is well known from studies on mammals (Slanger and Marchello, 1994; Gudivaka et al., 1996). Assuming that the benefits of non-lethal body composition estimates are related to the possibility of repeated measurements on individual fish over time at fluctuating temperatures, there is a need for temperature-dependent calibration of the assessment methods. The reliability of calibration results derived at a given temperature should ideally be high when applied to a different temperature in the field (Hartman et al., 2011).

The objectives of our study were to (i) compare the performance of K_n, BIA, and FM, to predict dry mass content as an indicator of energetic status using carp (*Cyprinus carpio*) and European eel (*Anguilla anguilla*) as model species, and (ii) to test for the effects of temperature on the functionality of BIA and FM. We choose carp as a recreationally and commercially important species in European fisheries and aquaculture (Arlinghaus and Mehner, 2003) and eel due to its currently declining status, which demands non-lethal assessments of energetic status to help understanding migration propensity or failure (Larsson et al., 1990). Both of these species have not undergone rigorous testing as to the suitability of BIA and FM. The only study published so far in carp has used FM readings and has reported positive correlations (Oberle, 2008), which underlines the hypothesis that at least FM should provide robust results in carp.

2. Materials and methods

Calibration for K_n, BIA and FM readings was conducted using $N = 80$ farmed scaled carp (Nordhauser Mühle, Ostercappeln, Germany, 52°19′53″ N, 8°14′51″ E) and $N = 40$ wild-captured yellow eel (Carl Peter Brasen eel export, Hemmet, Denmark). To increase the among-individual contrast in body composition of carp and eel and thus to increase the power of the calibration procedure, different feeding regimes were applied to the fish. Carp were kept under four different feeding regimes in aquaria ($N = 20$ carp in each treatment) for 117 days before measurements [Ø 5 mm commercial carp pellets, Trouw Nutrition carp pellets C-5, Trouw Nutrition, Burgheim, Germany; 0.2%, 1%, 2% and 4% of total body weight per day]. The aquaria (110 cm × 60 cm × 80 cm) were placed in a climate chamber with a standardized temperature of 20 °C and a light regime of 12:12 h. One third of the aquaria water was exchanged weekly and all tanks were continuously filtered using external filters (Eheim professional 3 type 2080, Eheim, Deizisau, Germany). Eel were kept in a circular laboratory tank (diameter 2 m). The tank was connected to a circulating water system and a biological filter. Light regime was 12:12 h. Water inflow was $1\,\mathrm{l\,s^{-1}}$ and water temperature ± SD was 15 ± 2 °C. After delivery, $N = 20$ individual eel were directly measured for their proximate body composition using BIA and FM. Another $N=20$ eel were starved within the circular tank for 45 days before subsequent analyses.

Prior to measurements, each fish was anaesthetized using a 1:9 clove oil/ethanol solution (0.75 ml l^{-1} water), excess water was removed with a paper towel and fish were then measured for their total length (TL, nearest mm) and weight (nearest g). Using length–weight relationships at the time of delivery for eel and after the feeding experiment for carp as reference (carp: mean TL 207.8 ± 18.5 mm, mean weight 126.8 ± 39.2 g; eel: mean TL 586.1 ± 93.1 mm, mean weight 318.9 ± 177.6 g), relative condition factors as described by Le Cren (1951) were calculated for each individual to test for the reliability of K_n to predict proximate body composition.

2.1. Sampling procedure BIA

For BIA measurements (serial resistance and reactance) a bioelectrical impedance analyzer was used (Quantum II; RJL Systems, Detroit, Michigan). The BIA system consisted of two sets of 3-gauge 10 mm long hypodermic needles. Each set included an outer transmitting and an inner detecting electrode held 1 cm apart in a plastic housing that allowed each needle to penetrate about 3 mm into the fish muscle. Electrodes were placed in the dorsal region of the fish following instructions by Cox and Hartman (2005). If necessary, 1–2 scales on the needle positions were removed in carp. Measurements took place on a non-conductive plastic board to avoid any current flow potentially biasing measurements (Cox et al., 2011). All BIA measurements were triplicated, and the distance between the two sets of needles was measured. Raw BIA measurements (resistance and reactance) and parallel-transformed raw BIA measurements (Pothoven et al., 2008) are cross-sectional measurements and should relate to relative tissue properties like relative dry mass (Rasmussen et al., 2012), whereas volumetric measurements (e.g. needle distance^2/parallel-transformed reactance, Cox and Hartman, 2005; Hanson et al., 2010) are three dimensional measurements and should reflect whole organism properties like total body water (Rasmussen et al., 2012). We calculated all of these parameters. Because there is no agreement whether parallel-transformed (Pothoven et al., 2008) or series-based (Rasmussen et al., 2012) measures of resistance and reactance should be used for calibration, mean values of the triplicated measures of both were used for analyses.

2.2. Sampling procedure fat meter

After BIA measurements, the microwave fat meter (MFM 992; Distell Inc., West Lothian, Scotland) was applied on the same region of the dorsal muscle. The FM sensor (frequency 2 GHz, power 2 mW) was placed along the dorsal surface of the fish at four positions for eel, and at one position for carp on both sides of the fish's body. Additional measurements on carp were not possible, because the FM microstrip sensor already covered most of the dorsal region. For measurements, the "carp-1" calibration and the "eel-1" calibration provided by the manufacturer were used. The manufacturer had previously conducted species-specific calibrations by chemical analyses of relative fat content of dorsal muscle tissue, which were then regressed against the FM readings (Distell, 2003). The device displays the result of this calibration as % lipids. Thus, species-specific settings provided by the manufacturer are most likely to provide the best results for dorsal muscle tissue, rather than for the whole body's nutrient composition (Crossin and Hinch, 2005). We applied the FM readings to predict individual's relative dry mass similar to the BIA approach. All measurements were triplicated on all positions. Mean values were then used in subsequent analyses.

2.3. Assessment of temperature-dependency

To test for the effects of temperature on the performance of BIA and FM in predicting relative dry mass, individuals were first measured at temperatures of the holding tanks and subsequently cooled down using iced water. Eel were initially measured at 15 °C and cooled down to 10 °C. Carp were initially measured at 20 °C and cooled down to 10 °C. While cooling, temperature of the fish within the body cavity was scanned using a digital slate thermometer (GTH 175/Pt, Greisinger electronic GmbH, Regenstauf, Germany), and BIA and FM readings were repeated once the target temperature was reached. During repeated BIA measurements, needle distances were held constant.

2.4. Laboratory analyses of proximate body composition

After BIA and FM measurements, anaesthetized fish were killed with an overdose of the anaesthetic (5 ml l^{-1} water) and whole bodies of $N=40$ eel and $N=40$ carp were homogenized using an electrical meat grinder (Krefft R-70, Krefft, Solingen, Germany) followed by grinding using a stirring staff (ESGE Zauberstab M100, ESGE AG, Mettlen, Switzerland). The remaining carp ($N=40$) were filleted and the white dorsal muscle was separated from bones and skin before homogenization to also calibrate BIA, FM and K_n for the dorsal muscle of carp. This was done to test if calibration of BIA and FM would in principle be possible using dorsal muscle tissue only. If so, non-lethal FM and BIA measurements could be taken in future applications in the field. Afterwards in the same fish, non-lethal muscle biopsies could be taken for calibration purposes, instead of killing the fish for subsequent laboratory analyses. Using this approach, few fish would die during the calibration process on purpose, which could be necessary if studies were to be conducted on protected species or rare specimens. Triplicated subsamples of homogenized tissue (4.3 ± 0.8 g) were used for laboratory analyses of dry mass, individual nutrients (lipids) and energy density (see for procedures below). Replicated homogenates were separately packed into plastic screw cap containers (40 ml) and stored at −80 °C until analyses.

To quantify water content and its inverse, dry mass, tissues were dried in a vacuum dryer (Zirbus technology GmbH, Bad Grunz, Germany) for 24 h at −20 °C and relative dry mass per individual wet mass was calculated. Due to malfunction of the vacuum dryer $N=18$ samples of whole body carp and $N=2$ samples of white dorsal muscle of carp were corrupted, resulting in a final sample size of $N=22$ for whole body carp and $N=38$ for white dorsal muscle of carp. Dry matter tissue lipids were extracted following the procedure outlined in Folch et al. (1957), and lipid content was calculated as percentage of dry mass. In addition, energy content of dry mass was estimated by bomb calorimetry (Parr 6400 Calorimeter, Parr, Frankfurt M., Germany) and energy density (kJ g^{-1}) of wet mass was then re-calculated considering the water content initially estimated per sample tissue.

2.5. Statistics

Initially, we conducted correlations between relative lipid content, energy density and relative dry mass in whole body samples of eel and carp and dorsal white muscle of carp using linear regressions. Subsequently, to derive relationships between our non-lethal measurements and observed relative dry mass of the sampled fish at 10 °C, 12 different multiple regression models were used. As independent variables the models contained (1) BIA derived serial resistance, serial reactance and needle distance, (2) BIA derived parallel-transformed resistance, parallel-transformed reactance and needle distance, (3) BIA derived volumetric reactance based on serial values, (4) BIA derived volumetric reactance

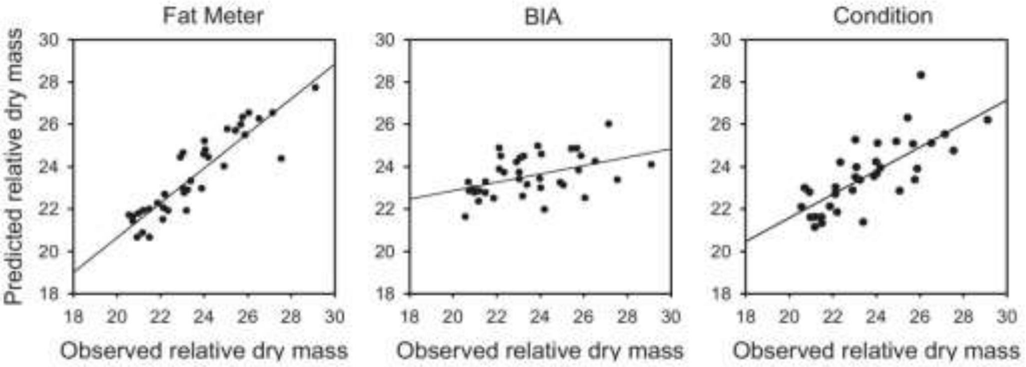

Fig. 1. Observed and predicted relative dry mass values for dorsal white muscle of carp derived from fat meter, cross-sectional parallel-transformed bioelectric impedance analyses (BIA) and relative condition factor.

based on parallel-transformed values, (5) FM readings, and (6) relative condition factor only. Models 7–12 were similar to models 1–6, but additionally included relative condition and total length (TL) as a covariate. To select the most parsimonious model, i.e. the best methodology to non-lethally assess dry mass of fish, second order Akaike information criteria for small sample sizes (AIC_c) were calculated (Burnham and Anderson, 1998). Afterwards the lowest AIC_c value was subtracted from the other AIC_c of alternative models to create a rank index referred to as $\Delta_i - AIC_{c(min)}$, where the best model has an index value of $\Delta_i - AIC_{c(min)} = 0$. To test for the dependency of needle distances on size of the fish in BIA measurements, measured needle distances were correlated with TL of the fish using Pearson's correlations.

To test for the effects of temperature on BIA and FM measurements in whole body carp we first compared BIA and FM outputs at 10 °C and 20 °C using paired T-tests. In addition, measurements taken at 20 °C were used to predict relative dry mass of the fish based on the calibrated regression model results at 10 °C. Thereby, we simulated researchers using calibrations from low temperature to predict dry mass of fish from samples taken at a higher temperature (20 °C). These predicted values for relative dry mass at 20 °C were then compared with laboratory-derived values using paired T-tests. Further, the same values for relative dry mass were tested for their rank order consistency using Spearman correlations. Statistics were conducted using SPSS 15.0 with an error probability of alpha = 0.05.

3. Results

3.1. Correlations between dry mass, lipid, and energy density in eel and carp

We found relative dry mass in whole bodies of eel and carp and in dorsal white muscle of carp to be significantly correlated with both relative fat content and energy density (all $P < 0.05$). Linear regression models explained 96.7% (whole body eel), 76.7% (whole body carp) and 88.9% (dorsal white muscle of carp) of the variance in relationships between relative dry mass and relative fat content, and 99.1% (whole body eel), 96.4% (whole body carp) and 97.2% (dorsal white muscle of carp) in relationships between relative dry mass and energy density ($kJ\,g^{-1}$). Thus, relative dry mass is a very useful proxy of the whole energetic status of eel and carp.

3.2. Suitability of non-lethal assessment methods for dry mass

The regression models used to calibrate BIA and FM for carp and eel without inclusion of TL and K_n revealed FM readings performing better in all cases and for both species compared to either BIA or the relative condition factor K_n (Table 1). Differences between models using series-based and parallel-transformed BIA measurements as independent variables were negligible and therefore, only results based on parallel-transformed values are presented. Significant linear relationships with high predictive power (67.6–81.4%)

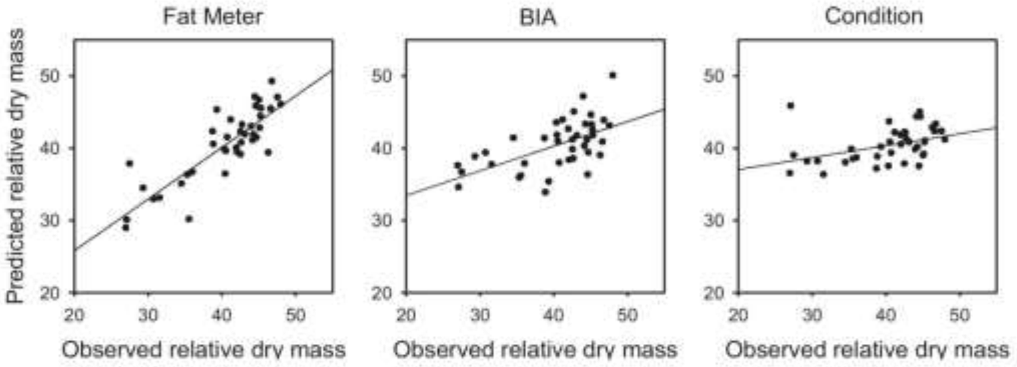

Fig. 2. Observed and predicted relative dry mass values for whole body eel derived from fat meter, cross-sectional parallel-transformed bioelectric impedance analyses (BIA) and relative condition factor.

Table 1

Regression models derived from calibration of FM, BIA and K_n for predicting relative dry mass (DM) at 10 °C. Predictor variables are fat meter readings (FM), relative condition factor (K_n), parallel-transformed resistance (R_P), parallel-transformed reactance (X_P), needle distance (D_N), volumetric reactance based on parallel-transformed values (D_N^2/X_P), and total length (TL).

Species	Model	Standardized coefficients	R^2	P	ΔAIC$_c$
Cyprinus carpio dorsal muscle	$DM = 16.66 + 0.70(FM)^*$	$0.91(FM)$	0.814	**<0.001**	0.0
	$DM = 9.24 + 15.18(K_n)^*$	$0.75(K_n)$	0.544	**<0.001**	24.9
	$DM = 20.81 + 1.37(D_N^2/X_P)^*$	$0.43(D_N^2/X_P)$	0.161	**0.008**	51.1
	$DM = 20.13 - 0.10(R_P) + 0.00(X_P) + 0.15(D_N)^*$	$-0.11(R_P), -0.01(X_P), 0.47(D_N)$	0.124	0.061	72.0
	$DM = 12.84 + 0.58(FM)^* + 3.20(K_n) + 0.01(TL)$	$0.76(FM), 0.16(K_n), 0.07(TL)$	0.820	**<0.001**	3.2
	$DM = -9.35 - 0.01(R_P) + 0.00(X_P) - 0.11(D_N) + 14.57(K_n)^* + 0.08(TL)$	$0.15(R_P), 0.21(X_P), -0.33(D_N), 0.72(K_n), 0.58(TL)$	0.576	**<0.001**	39.5
	$DM = 4.92 + 13.77(K_n)^* + 0.03(TL)$	$0.68(K_n), 0.22(TL)$	0.575	**<0.001**	28.9
	$DM = 3.90 - 0.16(D_N^2/X_P) + 13.82(K_n)^* + 0.03(TL)$	$-0.05(D_N^2/X_P), 0.68(K_n), 0.20(TL)$	0.562	**<0.001**	28.8
Anguilla anguilla whole body	$DM = 23.10 + 0.61(FM)^*$	$0.85(FM)$	0.707	**<0.001**	5.1
	$DM = 8.14 + 0.00(R_P) + 0.00(X_P) + 1.45(D_N)^*$	$0.04(R_P), 0.59(X_P), 0.68(D_N)$	0.285	**0.002**	56.6
	$DM = 23.65 + 16.65(K_n)^*$	$0.41(K_n)$	0.143	**0.009**	33.9
	$DM = 37.87 + 71.87(D_N^2/X_P)$	$0.23(D_N^2/X_P)$	0.028	0.152	33.5
	$DM = 9.75 + 0.55(FM)^* + 7.98(K_n)^* + 0.01(TL)^*$	$0.77(FM), 0.19(K_n), 0.19(TL)$	0.763	**<0.001**	0.0
	$DM = -49.57 + 0.01(R_P)^* + 0.00(X_P)^* - 2.64(D_N)^* + 23.42(K_n)^* + 0.14(TL)^*$	$0.50(R_P), 0.51(X_P), -1.24(D_N), 0.57(K_n), 2.17(TL)$	0.665	**<0.001**	29.2
	$DM = -23.53 - 369.6(D_N^2/X_P)^* + 24.61(K_n)^* + 0.09(TL)^*$	$-1.19(D_N^2/X_P) + 0.60(K_n) + 1.40(TL)$	0.342	**<0.001**	17.5
	$DM = 12.76 + 16.49(K_n)^* + 0.02(TL)^*$	$0.40(K_n), 0.30(TL)$	0.212	**0.005**	41.4
Cyprinus carpio whole body	$DM = 21.04 + 1.07(FM)^*$	$0.83(FM)$	0.676	**<0.001**	3.1
	$DM = 1.80 + 0.14(R_P)^* - 0.01(X_P)^* + 0.01(D_N)$	$0.73(R_P), -0.35(X_P), 0.01(D_N)$	0.552	**<0.001**	24.0
	$DM = 24.65 + 3.38(D_N^2/X_P)^*$	$0.34(D_N^2/X_P)$	0.261	**0.007**	14.5
	$DM = 6.86 + 27.56(K_n)^*$	$0.52(K_n)$	0.234	**0.011**	13.7
	$DM = 21.07 + 1.12(FM)^* - 16.49(K_n) + 0.07(TL)$	$0.87(FM), -0.31(K_n), 0.30(TL)$	0.707	**<0.001**	0.0
	$DM = -58.72 + 0.17(R_P)^* - 0.02(X_P) - 0.68(D_N)^* + 10.03(K_n) + 0.32(TL)^*$	$0.74(R_P), -0.05(X_P), -1.05(D_N), 0.19(K_n), 1.36(TL)$	0.661	**<0.001**	15.0
	$DM = -21.56 - 3.74(D_N^2/X_P) + 14.20(K_n) + 0.23(TL)$	$-0.60(D_N^2/X_P) + 0.27(K_n) + 0.08(TL)$	0.330	**0.014**	7.9
	$DM = -0.56 + 9.93(K_n) + 0.11(TL)$	$0.19(K_n), 0.48(TL)$	0.330	**0.007**	14.8

Bold P values indicate significant regression models.

* Significant coefficients ($P < 0.05$).

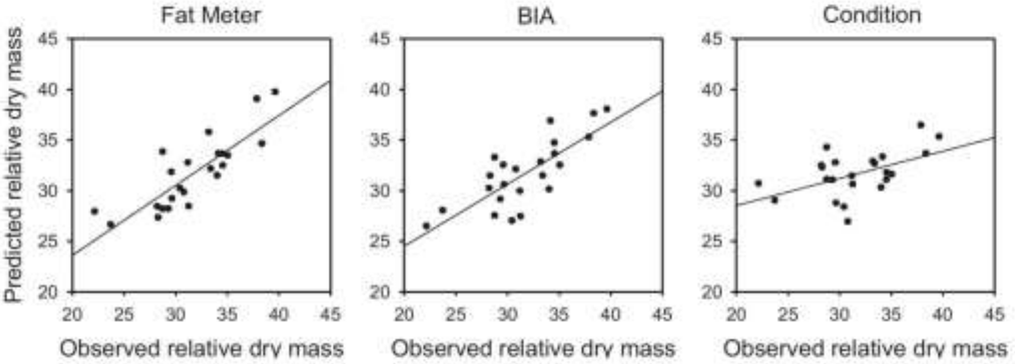

Fig. 3. Observed and predicted relative dry mass values for whole body carp derived from fat meter, cross-sectional parallel-transformed bioelectric impedance analyses (BIA) and relative condition factor.

were found between FM readings and laboratory-derived values for relative dry mass in all cases (Table 1, Figs. 1–3). Significant relationships were also found between K_n and relative dry mass of the fish, but the degree of variances explained was comparably low (14.3–54.4%; Table 1, Figs. 1–3). Compared to FM, all BIA outputs were found to perform similarly to K_n-based models when predicting dry mass of eel and carp (Table 1, Figs. 1–3). Comparisons of volumetric BIA measurements and cross-sectional BIA measurements revealed inconsistent results (Table 1). However, in most cases, parallel-transformed cross-sectional BIA measurements were not significant (Table 1), indicating a low contribution of these variables to the degree of explained variances. By contrast, needle distance and volumetric measurements including needle distances were found to be significant in most cases (Table 1), and needle distances were highly correlated with the size of the fish (Pearson correlation, $r = 0.964$, $P < 0.001$). This suggests that length of the fish rather than BIA measurements itself might have been responsible for the somewhat small predictive power of the BIA device. This assumption was supported by the models including TL and K_n. Explained variances in BIA models increased substantially after inclusion of these variables (57.1–66.5%; Table 1), whereas FM and K_n models only slightly changed (Table 1). In addition, AIC_c values of BIA and K_n models differed substantially from those of the FM models, indicating generally higher reliability of FM measurements compared to both K_n and BIA measurements.

3.3. Effects of temperature on non-lethal dry mass assessments

We found BIA measurements to be significantly temperature-dependent using whole body carp (mean parallel-transformed resistance at 10 and 20 °C ± SD 305.3 ± 23.0 and 257.9 ± 36.7 and mean parallel-transformed reactance at 10 and 20 °C ± SD 965.0 ± 124.7 and 809.4 ± 137.1, respectively, paired T-tests, $N = 22$, $T = 7.4$, $P < 0.001$ and $N = 22$, $T = 14.1$, $P < 0.001$), whereas FM readings did not differ between 10 and 20 °C (mean FM readings at 10 and 20 °C ± SD 9.9 ± 3.1 and 10.0 ± 3.4 respectively, paired T-test, $T = 0.3$, $P = 0.875$). Predicted values of mean relative dry mass were found to be significantly lower than those achieved under laboratory conditions when applying BIA measurements at 20 °C for dry mass content estimation using the model calibrated to 10 °C (mean relative dry mass measured under laboratory conditions and mean calculated relative dry mass for measurements at 20 °C 31.7% ± 4.3% and 29.7% ± 3.1% respectively, paired T-test, $T = 2.7$, $P = 0.013$; Fig. 4). Therefore, one would underestimate dry matter content at warm water if a model calibrated to cooler water is used. This systematic bias did not change the rank order of dry matter content across individuals (Spearman correlation, Spearman's rho = 0.498, $P = 0.016$), but biased the absolute estimated value downwards. No such contrast was conducted for whole body eel and dorsal white muscle of carp because the initial calibration results for BIA measurements were not significant, suggesting no predictive power.

4. Discussion

Our analyses showed that FM consistently performed better than BIA and K_n to predict relative dry mass as an index of energetic density in whole body eel and carp and dorsal white muscle of carp. These results confirmed that highly reliable non-lethal dry mass estimations are possible using a FM in fish species that are comparably rich in lipids such as eel and carp. Due to the correlation of dry mass and lipid content, and particularly energy density, the FM approach can thus be considered useful for predicting the energetic status of eel and carp. Our results, however, raise a cautionary note as to the usefulness of relative condition metrics and BIA assessments to infer insights about the energetic status in these species. We are not claiming that the use of BIA and K_n is superfluous in fish in general, as indeed some weak correlation between measures and relative dry mass were found in our study species. Instead, we contend that in carp and eel, FM based models will outperform BIA models, and thus, BIA is inferior to FM in these species.

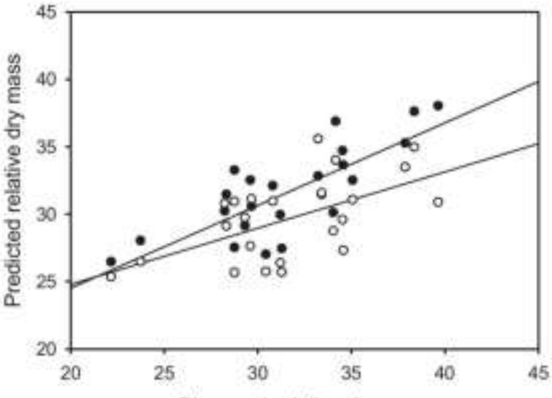

Fig. 4. Temperature dependency of cross-sectional parallel-transformed BIA measurements for whole body carp. Black dots indicate calibration results at 10 °C and white dots indicate calculated values of relative dry mass at 20 °C based on calibration results at 10 °C.

Our study was confined to predict relative dry mass, based on the fact that both BIA and FM directly measure water content as the inverse of dry mass (Schoeller, 2000). Dry mass is a suitable index of the energetic status of fish if it is strongly correlated with energy density or with stored energy, which is closely related to lipid content in most species that are rich in fat (Chellappa et al., 1995; Schreckenbach et al., 2001). As expected, we found relative fat content and energy density to be highly correlated with relative dry mass. Thus, relative dry mass can be used as a proxy for relative fat content and energy density in fish. We propose that the simplest index value of energetic status that may be estimated even from small tissue samples (such as those stemming from non-lethal muscle biopsies) is tissue dry matter. In this context, FM is useful as a non-lethal assessment tool to infer dry mass of fish. Therefore, future calibration studies could be confined to relative dry mass only without loss of information (see also Jonas et al., 1996). It is straightforward to conduct FM on a live fish and then a rapid muscle biopsy that is later dried in the laboratory to assess dry mass. This way, potentially spurious and more variable correlations between specific body constituents such as protein and ash and BIA and FM readings are avoided and more importantly, time and cost intensive laboratory analyses can be cut down and the fish remains alive.

Our findings contrast with previous work in several fish species using BIA who reported strong relationships between BIA outputs and proximate body composition (e.g., Cox and Hartman, 2005; Duncan et al., 2007; Hanson et al., 2010). In contrast to our work, these studies involved the regression of BIA measurements on absolute values of proximate body composition rather than relative values. Pothoven et al. (2008) compared calibration results for BIA measurements between total and relative body composition values. Similar to our work, they found weak relationships for relative values of body composition, and strong ones for absolute values. All above mentioned studies used volumetric impedance measures in their regression analyses. Because this three-dimensional approach reflects the mass of water within the electric field induced by BIA (Liedtke, 1997), strong correlations between total mass of body constituents and volumetric BIA measurements are to be expected (Rasmussen et al., 2012). However, the volumetric approach is confounded by the TL of the fish or needle distances (Cox and Hartman, 2005; Hanson et al., 2010). We found both variables to be highly correlated, if needles are placed along morphological landmarks as outlined in Cox and Hartman (2005). Therefore, needle distances are just a proxy of fish size and strong correlations between BIA and proximate body composition may just be caused by the co-variance of absolute body composition with fish length. Similar to the volumetric approach, analyses using cross-sectional BIA measurements commonly include measures of size (Pothoven et al., 2008) or weight (Bosworth and Wolters, 2001) of the fish as co-variate. Due to the strong relationship between size and absolute values of nutrients carried by an individual (Caulton and Bursell, 1977; Weatherley and Gill, 1983), strong relationships can be expected in joint models involving size or weight and cross-sectional BIA outputs. Non-lethal assessments of proximate body composition can be considered most valuable if measurements directly correlate with relative dry mass of the fish. Although the inclusion of TL and K_n in regression models strongly increased the explanatory power of BIA in our study, high variance explanation seems to be unrelated to the BIA measurements per se. Consequently, BIA might be of less use than claimed before, and it was indeed found to be of low utility in carp and eel in our study.

From an ecological perspective it is most important to discern among-individual differences in relative body constitution, because this reflects the potential for fitness differences or past foraging success. In this context, in our study BIA measurements did not work to a degree that renders the device of use in the field. This finding contrasts with the results presented by Hartman et al. (2011) and Rasmussen et al. (2012). Hartman et al. (2011) combined dorsal and ventral BIA measurements in their analyses, and they used several electrical equations to calculate a broad range of candidate predictor variables for explaining relative dry mass of the fish. This approach led to up to ten variables within the same model, most of them relying on the same two measurements of resistance and reactance. Further, variables included in the regression models of Hartman et al. (2011) strongly differed between different size classes of the fish and with different temperatures at measurement. Thus, calibration results created by Hartman et al. (2011) cannot easily be compared with other studies without additional calibration work for the size classes of interest at a specific temperature. By contrast, measurement techniques between our study and Rasmussen et al. (2012) did not differ. Using parallel-transformed reactance as independent variable, Rasmussen et al. (2012) also found similarly weak relationships between BIA values and relative body water content as we did in carp and eel. Rasmussen et al. (2012) only observed strong relationships between relative body constituent values and BIA measurements when applying serial-based (i.e. raw) BIA measurements. This finding was not supported in our study where usage of serial-based and parallel-transformed values created negligible differences in the results of regression analyses. However, Rasmussen et al. (2012) used brook trout as a model species and calibration of BIA for relative body constituents might be species-specific. Although our study did not support the usefulness of simple BIA assessments to predict dry mass in eel or carp, it cannot be ruled out that combined dorsal and ventral BIA measurements, as conducted by Hartman et al. (2011), and/or application to different species can be a way to substantially improve the performance of BIA applications.

We found FM readings to be highly correlated with relative dry mass in all our investigated cases in both species, whereas the predictive power of relative condition-factor-only models for predicting relative dry mass was comparably low. Although linear regressions with K_n as independent variable were found to be highly significant, the degree of variance in dry mass explained by the condition-factor-only models were found to be substantially lower than those using FM as explanatory variable. Also, AIC$_c$ values were much higher compared to regression models based on FM indicating less supported models. Both, high reliability of FM readings for predicting energetic status of fish, and high uncertainty of relative condition factors for doing the same job are in agreement with previous studies (Kent, 1992; Vogt et al., 2002; Crossin and Hinch, 2005; Davidson and Marshall, 2010; Hanson et al., 2010). However, other studies found strong relationships between relative body composition values and relative condition factors (e.g. *Perca fluviatilis* Craig, 1977; *Morone saxatilis* Brown and Murphy, 1991), which contrasts with our results. Reasons for the lack of reliability of relative condition factors to predict energetic status in carp and eel in our study might have been caused by imprecision in calculating relative condition factors. Commonly, relative condition factors relate the actual weight to a calculated average weight using reference fish other than the subsample used for experimentation (Le Cren, 1951; Cone, 1989), whereas we used the same fish. Thus, our study does not generally discount the use of relative condition factors.

BIA measurements were significantly influenced by temperature, and measurements at 20 °C resulted in significantly different predictions for relative dry mass when calculated based on the calibration at 10 °C. No such dependency of FM measurements on temperature was observed, confirming previous studies that used energy meters on fresh and iced fish without any differences in FM readings (Vogt et al., 2002). Susceptibility of BIA measurements to fluctuating temperatures is known (Slanger and Marchello, 1994; Cox et al., 2011; Hartman et al., 2011), and reasons for this are likely based on differences in blood flow and velocity at different

Table 2
Strength and weaknesses of using bioelectric impedance analyses (BIA), fat meter (FM) and relative condition factor (K_n) to non-lethally assess the body composition of fish. "+" indicates strong support, "–" indicates low support, "NA" indicates insufficient knowledge.

Challenge/functionality	BIA	FM	K_n	References
Assessment of body moisture (water content) rather than direct assessment of proximate body constituents	+	+	–	Caulton and Bursell (1977), Kent (1992), and Schoeller (2000)
Reliability of measurements	–	+	–	Crossin and Hinch (2005), Pothoven et al. (2008), Hanson et al. (2010), and this study
Reliability at very high water levels	NA	–	+	Caulton and Bursell (1977), Distell (2003), and Crossin and Hinch (2005)
Reliability against temperature fluctuations	–	+	+	Kushner et al. (1996), Vogt et al. (2002), and this study
Calibration using muscle biopsy possible	+	+	–	Hendry and Beall (2004), and this study
Application to very small fish	NA	–	+	Caulton and Bursell (1977) and Kent (1992)
User experience needed	+	NA	–	Cox et al. (2011)

temperatures as it is known for humans (Kushner et al., 1996). Thus, even if BIA measurements can be calibrated successfully to other species than eel and carp as suggested by Hartman et al. (2011) and Rasmussen et al. (2012), application of the resulting regressions to the field would need to be done for a large gradient of temperature. This is particularly true if researchers want to benefit from the advantage of multiple measurements on the same individual over time and seasons, which usually will involve differences in ambient temperature. As shown in our study, predicted values for proximate body composition will be biased if researchers use calibration models from temperatures above or below actual measurements. Such biased estimates can be a source of concern if certain thresholds of lipid and energy density are ecologically relevant. For example, fish like carp are generally assumed to be in poor condition and likely do not survive harsh winters if energy density falls below 4 MJ/kg (Schreckenbach et al., 2001). Managers applying such threshold values might draw wrong conclusions, if measurements were taken at temperatures other than at calibration. The temperature-dependency is another argument to reject usage of BIA and to favour FM in species like eel and carp.

We showed that FM readings can be used to predict relative dry mass values of white dorsal muscle in carp, and calibration results were even better in this tissue than those for whole body of carp. Considering that FM only interacts with somatic tissue at a given location (Vogt et al., 2002), better calibration results in the white dorsal muscle are not surprising (Distell, 2003). Because we found FM readings to be highly correlated with relative dry mass, and relative dry mass to be strongly related to energy density in carp, high FM readings are indeed indicative of high energetic status of fish. Further, because lipids stored in white muscle can be correlated with whole body lipid content in fish (Viola et al., 1988; Regost et al., 2001), future calibration of FM readings might sometimes also be done using muscle biopsy without killing the fish (Hendry and Beall, 2004). This can be beneficial if the study objects belong to a protected species or killing of individuals is impossible. However, in cases where body constituents are stored along a head–tail gradient, as it is known for some migrating salmon (Herbinger and Friars, 1991), multiple biopsy samples might be necessary.

A range of authors have discussed advantages and disadvantages of BIA and FM to non-lethally infer indices of condition and energetic status, or even proximate body composition in fish (Table 2). Based on the existing literature (Table 2) and the results of our study, we conclude that FM provides the most robust and repeatable results, unless lipid contents are too low [~2.5% body fat content (Crossin and Hinch, 2005); Table 2]. Also, compared to BIA, the FM device is less prone for misapplication based on inexperience (Cox et al., 2011). Nevertheless, applicants are recommended to test the data generation in the field, and pay attention to the angle by which the FM device is placed on the tissue of interest. Correct angles can be trained using a calibration tool that is provided with the device. Also, triplicated readings are encouraged to minimize measurement error. However, in cases where the reading head of the FM is bigger than the dorsal region of the fish, the FM technique cannot be used anymore (Table 2). For such cases we propose relative condition factors as an alternative (Table 2).

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Paper IV

IV

Thomas Klefoth, Philip Rebensburg, Christian Skov, Anna Kuparinen, Robert Arlinghaus, unpublished. Towards a mechanistic understanding of vulnerability to hook-and-line fishing – the role of behavior as a basis for understanding angling-induced evolution. Submitted to Evolutionary Applications (05.09.2015) and invited for resubmission.

Towards a mechanistic understanding of vulnerability to hook-and-line fishing – the role of behavior as a basis for understanding angling-induced evolution

Abstract

In passive fisheries, boldness-related behaviors might fundamentally affect vulnerability of fish. Hence, boldness should be under strong selection. We used juvenile common-garden reared carp (*Cyprinus carpio*) within a narrow size-range to investigate the mechanistic basis of selection caused by angling. We focused on one key personality trait (boldness; measured individually within the laboratory and in groups in ponds), two morphological traits (body-shape and head-shape), one life-history trait (growth capacity), and intrinsic hunger levels (energetic status). Carp behavior within the laboratory was unrepeatable, but highly consistent within ponds. Laboratory boldness, energetic status and body-shape were not or only weakly related to angling vulnerability. Contrary, boldness in ponds turned out to be the most important trait under selection, followed by juvenile growth. We found juvenile growth to be only moderately correlated with boldness in ponds. Hence, direct selection on boldness will also induce indirect selection on juvenile growth, but independent evolution of both traits is also possible. Our study is among the first to mechanistically reveal that energy-acquisition related behaviors, and not growth rate per se, are key factors determining the probability of capture and subsequent death by removal. We predict an evolutionary response towards reduced boldness in highly angling-exploited fish stocks.

Introduction

A growing body of literature has drawn attention to the potential for intensive and/or size-selective fisheries to act as an evolutionary force altering a range of life-history traits such as reproductive investment, size and age at maturation and genetic growth capacity (reviewed in Law 2000, Heino and Godø 2002, Jørgensen et al. 2007, Kuparinen and Merilä 2007, Laugen et al. 2014). Most work on harvesting-induced evolution in a fisheries context has focused on experimentally harvested fishes under laboratory conditions (e.g., Conover and Munch 2002, Uusi-Heikkilä et al. 2015) and on time series analyses of phenotypic data in commercial fisheries settings (e.g., Olsen et al. 2004, Swain et al. 2007). Some recent studies have also addressed the question of fisheries-induced adaptive changes in the context of recreational fishing, largely confirming the findings of expected changes in life-history traits. Accordingly, intensive and/or size-selective recreational fishing has been found to lead to increased reproductive investment and reduced age and size at maturation, which collectively reduces adult size-at-age (Arlinghaus et al. 2009, Saura et al. 2010, Matsumura et al. 2011, Alós et al. 2014a). Moreover, work in largemouth bass (*Micropterus salmoides*) selected for high and low vulnerability to angling has revealed genetically based changes in behavioral traits such as aggression and vigilance during parental care (Philipp et al. 2009, Sutter et al. 2012). It is likely that other fishes will also evolutionary adapt to high angling pressure by modifying life-history and behavioral traits, but other than for life-history traits, clear documentation of evolution of behavioral traits is still missing (Heino et al. in press).

The use of passive fishing gear such as hook-and-line, gill nets or traps is common worldwide in recreational fisheries (Arlinghaus and Cooke 2009). Passive fishing gear should directly select on behavioral traits related to exploration, activity, boldness or aggression because these traits directly affect exposure of individual fish to the fishing gear by increasing encounter probabilities (Heino and Godø 2002, Biro and Post 2008, Uusi-Heikkilä et al. 2008, Phillip et al. 2009, Enberg et al. 2012, Olsen et al. 2012, Diaz Pauli et al. 2015). Direct selection on behavioral traits can also indirectly change growth rate and other life-history traits as long as these traits are heritable and positively correlated with the behavioral trait under selection (Biro and Post 2008, Uusi-Heikkilä et al. 2008, Enberg et al. 2012). Although strong selection pressures acting on behavioral traits in recreational fisheries are clearly supported by theoretical arguments and simulation models (Uusi-Heikkilä et al. 2008, Alós et al. 2012, Enberg et al. 2012), only few experimental studies on this topic exist so far. The majority of these support the assumption of positive correlations between exploration, aggression or boldness and vulnerability to the passively operated hook-and-line fisheries (Cooke et al. 2007, Sutter et al. 2012, Klefoth et al. 2013a, Härkönen et al. 2014, 2015, Alós et al. 2015). In other passively operated gear types, such as traps or gill nets, a greater vulnerability of active and bold individuals has also been reported (Wilson et al. 1993, Biro and Sampson 2015). We thus expected a very pronounced relationship of fish personality and vulnerability to hook-and-line fisheries.

Most fishing gears typically operate positively size-selective for physical (gape size) and managerial reasons (size-based harvest limits) (Lewin et al. 2006, Garcia et al. 2012). Size-selective harvesting as it is common in most fisheries has prompted the "intuition" (Walters and Martell 2004) that fisheries-induced evolution of slow growth should be expected in most exploited situations. Indeed, the heritability of growth rate is at least moderate in fishes (Gjedrem 1983, Garcia de Leaniz et al. 2007) and therefore, selective harvesting of the fast growing portion of a fish population over several generations could lead to evolutionary downsizing (Conover and Munch 2002, Edeline et al. 2007, Swain et al. 2007, Matsumura et al. 2011, Alós et al. 2014a). However, depending on the local fitness landscape and the relationship of size and/or growth rate and natural fitness (Carlson et al. 2007), strong natural selection pressures favoring large body size can nullify any negative selection differentials induced by fisheries (Dunlop et al. 2009, Matsumura et al. 2011, Enberg et al. 2012) and maintain fast growing fishes in the face of exploitation (Edeline et al. 2007). Moreover, adult body size will not only be influenced by growth because adult growth is strongly affected by changes in maturation timing. Changes in adult body size may thus mechanistically be caused by alterations of the age and size at maturation and the investment into reproduction (Heino et al. 2008). Modelling of recreationally exploited stocks also showed that the selection differentials on growth rate can range from negative to positive depending on the selective properties and the intensity of harvesting (Matsumura et al. 2011). However, the strength and direction of selection acting on multiple life-history traits will likely be stock- and fishery-specific, which makes general predictions about expected phenotypic changes induced by harvesting inherently difficult (Dunlop et al. 2009, Matsumura et al. 2011, Enberg et al. 2012, Laugen et al. 2014).

There is some conceptual confusion in the fisheries literature with regard to the underlying mechanistic basis of observed changes in adult growth rate in time-series analysis (Enberg et al. 2012). Mechanistically, observed changes in adult growth rate can be a consequence of altered maturation schedules due to fisheries evolution of lower age and size at maturation and elevated reproductive investment, changes in genetic growth capacity must not be involved (Heino et al. 2008, Enberg et al. 2012, Alós et al. 2014a). Obviously, changes in adult growth rate may also be caused by fisheries-induced evolution of juvenile growth rate, but it is important to understand the mechanistic basis of changes in juvenile growth rate (Enberg et al. 2012). Key processes involved in energy budgeting are behavior-based energy acquisition, surplus energy allocation and somatic growth (Enberg et al. 2012). High energy acquisition caused by intensive foraging will foster fast somatic growth rates in juvenile fish (Heino et al. 2008, Lester et al. 2014). Because no energy is channelized into gonad tissue in juveniles, their growth rate constitutes a clean measure of growth capacity in fishes. However there is limited evidence for fisheries-induced evolution of slow juvenile growth rate in the wild (Enberg et al. 2012, but see Evangelista et al. 2015). A recent study in experimentally fished crayfish showed that trapping selectively captured fast growing juvenile crayfish and that fast growth was strongly correlated with boldness (Biro and Sampson 2015). Hence, selection on juvenile growth may be directly caused by selection acting on behavior.

Energy-acquisition related behaviors are likely to play a fundamental role in determining vulnerability to recreational fishing because the probability of capture is strongly related to the amount and location of food ingestion as well as the propensity to find, approach, attack or ingest a lure or bait. Therefore, evolutionary changes in adult growth rate reported in response to intensive recreational angling (e.g., Saura et al. 2010) might be an indirect consequence of direct selection acting on resource-acquisition-related behaviors (e.g., sensing and finding food, searching for food, feeding in the presence of predators, Biro and Post 2008, Uusi-Heikkilä et al. 2008, Enberg et al. 2012). One of the key resource-acquisition related behaviors in fishes is boldness, which is considered a personality trait defined as risk-taking behavior in non-novel environments (Réale et al. 2007). Although one would expect recreational angling to directly select on boldness-related behaviors in fish, the evidence for this is mixed in the literature (for a negative finding see Wilson et al. 2011, for a positive finding see Klefoth et al. 2013a).

Consistent variation in behavior within and among individuals is defined as personality or temperament (Realé et al. 2007), which has been shown to have a strong genetic basis in fishes (Dochterman et al. 2015). . However, revealing the personality of individual fish in standardized laboratory test environment has proved to be challenging in some species (e.g., Toms et al. 2010, Klefoth et al. 2012, Biro and Stamps 2015). For example, genotype by environment interactions, housing induced stress as well as strong habituation effects can significantly bias the behavioral expressions shown by individual fish and obscure any personality differences that might in fact be present in the wild (Klefoth et al. 2012). Hence, between individual variation may not always translate from the wild into the test environment in the laboratory (Niemelä and Dingemanse 2014). When assessing the correlation of behaviors and vulnerability to capture, behavioral measurements taken within controlled laboratory environments should thus be interpreted with caution, and ideally laboratory assessments should be paired with field validations to infer the presence of personality (Klefoth et al. 2012, Niemelä and Dingemanse 2014) and robustly test the relationship of personality and vulnerability to fishing.

In addition to behavior and potentially life-history, morphological and a range of state variables (e.g., hunger level) can also affect the likelihood of capture and therefore contribute to the selective properties of recreational fishing. For example, Alós et al. (2014b) found that more streamlined coastal fish and fish with larger mouth gaps were more likely to be captured by rod and reel angling than deeper bodied fish and fish with small mouth gaps. These findings could represent correlations of body shape and swimming activity (Haas et al. 2015) or relate to physical aspects of foraging in relation to hook size and gape-size limitations. Klefoth et al. (2013a) and Mezzera and Largiadèr (2001) also reported that more domesticated carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) showed higher propensities of recapture after release compared to wild fish. In carp, domestication is related to increased body depth (Matsuzaki et al. 2009), which is also known to be a morphological surrogate for the many changes in physiology and behavior that are related to the domestication process (Suzuki et al. 1978, Huntingford 2004).

In addition to morphological variables that integrate the ecology of the fish over longer periods of time, also state variables will influence vulnerability to capture. For example, poorly nourished and generally hungry fish are more likely to take risks (Godin and Crossman 1994) and thus should be more likely captured by hook-and-line fisheries like longlining (Løkkeborg et al. 2014) and angling (Heermann et al. 2013) than satiated fish. Hungry fish will have a higher feeding motivation and a lower degree of wariness (Godin and Crossman 1994, Stoner 2004). Based on this background we expected that behavioral, morphological and life-history traits as well as energetic status should jointly determine the vulnerability of individual fish to passively operating angling gear.

In the present study, we used juvenile carp (*Cyprinus carpio*) of identical age and a narrow size range as a model species to test for the strength and direction of selection acting on boldness-related behaviors, growth, morphological characteristics and nutritional status of the fish in a passive angling fishery. Our specific objective was to shed light on the behavior-based mechanisms underlying vulnerability to angling and to disentangle the relative importance of behavior and growth. From a mechanistic perspective, it was hypothesized that resource-acquisition related behaviors constitute key traits under selection in passively operating angling fisheries for carp and that accounting for boldness captures a relevant portion of direct selection acting on body size or growth rate similar to the crayfish study by Biro and Sampson (2015). Our research differed from Biro and Sampson (2015) due to the use of comparatively large semi-natural pond environments where the encounter rates of the individual carp with the angling gear should be less direct than in laboratory-based studies. Our study thus bridged the link between controlled laboratory studies and wild-like conditions, thereby improving the transferability of study findings to real fisheries.

Material and Methods

We performed two experiments aimed at quantifying capture-probability related selection gradients on key behavioral and morphological traits as well as juvenile growth rate and nutritional status in recreational angling using juvenile carp as a model species. To derive consistent behavioral traits that characterize the personality (e.g., boldness) of individual carp, in experiment 1 N = 96 fish were tested for their behavior within different experimental contexts in a controlled laboratory setting as it is typical in fish personality research (Toms et al. 2010). Afterwards, standardized pictures were taken for analyses of geometric morphometrics, and non-lethal measurements of body lipid content were conducted using a validated energy meter (Klefoth et al. 2013b). Afterwards, boldness-related behavioral traits were re-assessed after release in three replicated semi-natural ponds in a group context, followed by seven days of angling (experiment 1). To further investigate the vulnerability of our test fish to passive angling tactics in a longer term, pond experiments were repeated using N = 120 new fish from the same cohort (experiment 2), but with a longer angling period (7 and 20 days of angling rather than only 7 days of angling in experiment 1). Given the longer angling duration in experiment 2, we also measured growth rate of the experimental fish in the ponds.

Experimental fish

All carp were raised at a commercial fish hatchery (Fischzucht Wegert, Ostercappeln, Germany, 52°19'52'' N, 8°14'48'' E) in the same common garden pond environment. About 40 phenotypically scaled parental carp were stocked into a monoculture pond in spring. Spawning and breeding occurred naturally. The emerging young-of-the-year carp consisted of scaled and mirror carp phenotypes and were fed with standard carp dry food (1-3 mm diameter, Aller Classic, Aller Aqua, Golßen, Germany) in addition to natural food ingested in the shallow (1.5 m deep) earthen breeding pond (40 m x 50 m). This pond was fed with water from a nearby creek (Caldenhofer Graben). When the fish reached an age of about 10 months, the pond was drained and a random sample of scaled and mirror carp phenotypes was transported to the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin, Germany. There, fish were initially kept in indoor tanks (1 m x 1 m x 1 m, 5 fish per 100 l) fed with tap water (mean temperature ± SD 18 ± 1.5° C, exchange rate once per day) for five weeks until experiments started. Fish were exclusively fed with standard carp pellets (5 mm diameter, Aller Classic, Aller Aqua, Golßen, Germany) at a maintenance ratio of about ~1.5 % of fish body wet mass per day. Before experiments started, fish were slowly acclimatized to water temperatures within the test environments (aquaria or ponds) by altering the temperature at a maximum of 1 °C per day (Pitt et al. 1956). The maximal total change in temperature the fish experienced over the acclimatization period was 3 °C.

Individual assessment of personality of carp in the laboratory (experiment 1)

In an attempt to derive robust measures of the personality of individual carp, variants of an open field test known to measure boldness in fish were conducted (Fig. 1). We used individual focal fish under fully standardized conditions as it has been done successfully with juvenile carp before (Huntingford et al. 2010). Experimental carp were investigated for their exploration behavior, boldness and sociability within four different setups, which were replicated once per individual. The exploration test was conducted first at the beginning of a week (Monday) followed by a random order of the other three contextual trials where one trial was performed per day (Tuesday-Thursday) (see Appendix 1 for full description). The exploration trial was similar to an open-field test (Walsh and Cummins 1976). Here the time elapsed until the fish left the refuge was measured (Fig. 1). In addition, the time spent within the upper water zone was assessed as an initial boldness predictor. Within the other three contexts, boldness was supposed to be indicated by the time until feeding in an open field and after disturbance, the time spent exploring a novel object and the time spent shoaling with conspecifics (Fig. 1, Appendix 1). In every test, fish were given 60 minutes to leave the refuge. Afterwards a 10 minute observation period started (see Appendix 1 for full description).

The assessment of carp personality under laboratory conditions was conducted in two steps. First, a randomly selected subsample of the fish (N = 36, mean TL ± SD 136 ± 8 mm) was tested within all four contextual trials twice. In order to test for the consistency of any among individual variation in behavior, all four trials were repeated exactly six weeks after the first trials, because between trial periods can affect the repeatability of behaviors in fish and longer periods are more reliable and reduce issues of covariance of behaviors when assessed in short time periods (e.g., two days apart) among trials (Bell et al. 2009). Afterwards, the initial data were analyzed for evidence of the existence of repeatable and consistent personality traits within the first N = 36 focal carp. Unfortunately, these analyses revealed strong evidence for habituation effects leading to inconsistent and unrepeatable behavior culminating in the inability to identify robust signals for personality in our carp under laboratory conditions (Appendix 1). Based on this initially unexpected outcome (but see Klefoth et al. 2012 for supporting evidence that laboratory trials are not able to identify personality in carp in group settings), the remaining fish (N = 60, mean TL ± SD 171 ± 15 mm) were only tested once in a second step, and experiments were not repeated anymore after six weeks. The second subsample of fish was tested for their exploration behavior in a novel environment and their boldness-related behaviors in the context of foraging (time until feeding in an open field and after disturbance). We thereby strictly followed the previously established protocols for these two trials (Appendix 1). We focused on these two traits because both exploration and boldness should relate to vulnerability to angling (Uusi-Heikkilä et al. 2008).

All laboratory experiments were conducted within a temperature-controlled climate chamber (ILKAZELL, inner dimensions: 276 cm x 210 cm x 176 cm, ILKAZELL Isoliertechnik GmbH, Zwickau, Germany) at 20 °C to standardize environmental conditions and to control for effects of temperature on the expression of personality in fish (Biro et al. 2009). Two weeks before experimentation, all fish were equipped with a PIT tag (12 mm, Trovan, Weilerswist, Germany) for individual identification following the procedure outlined in Skov et al. (2005).

Assessment of personality, morphology, energetic status and vulnerability to intensive short-term angling in ponds (experiment 1)

After the personality assessment in the laboratory, new behavioral observations with the same laboratory fish released in randomly selected groups into three replicated experimental ponds were conducted. These experiments were designed to assess the boldness-related personality of angling-naïve carp in a semi-natural environment in groups, which has previously been found to yield reliable data in carp (Klefoth et al. 2012). Before release, we surgically implanted a new set of PIT tags (23 mm length, 2 mm width, Oregon RFID, Oregon, USA) into the fish`s body cavity. All ponds were equipped with PIT tag antenna loops (Oregon RFID, Oregon, USA) that were able to detect the newly inserted PIT tags (Fig. 1, Appendix 2). During PIT tag surgery, fish were anaesthetized using 1 ml l^{-1} of 9:1 solution of ethanol:clove oil in well aerated water at 18° C. After surgery, fish were measured for total length (TL, to the nearest 1 mm), and standardized pictures were taken from both sides of the fish`s body for geometric morphometrics analyses (Nikon DX40 mounted approximately 45 cm above the fish on a fix stand). Before pictures were taken, fish were placed in a straight position and the fins were stretched. Further, the body lipid content of the fish was non-lethally assessed using a validated energy meter (MFM 992, Distell Inc., West Lothian, Scotland, frequency 2 GHz, power 2 mW, setting "carp-1") following the procedures outlined by Klefoth et al. (2013b) to estimate the energetic status of the fish at release.

Stationary passive telemetry systems within 3 replicated experimental ponds (12 m x 5 m x 1 m, L x W x H, Fig. 1) were simultaneously used to enumerate carp behavior in groups. Each of three ponds were stocked with 32 randomly selected carp (mean TL ± SD pond 1: 178 ± 17 mm, pond 2: 176 ± 15 mm, pond 3: 177 ± 21 mm) tested for their behavioral consistency in the laboratory two month before release into the ponds. Carp were held in monoculture with no fish predators, but ponds were regularly visited by fish-eating birds. The ponds were continuously supplied with unfiltered lake water (about 1 l s^{-1}) from the nearby Müggelsee in Berlin (52°26'57'' N, 13°38'59'' E). A shelter structure [rectangular area of the pond (2 m x 5 m)] made out of black plastic and two open feeding spots (0.5 m diameter) in different distances to the shelter were installed (see Fig. 1 and Klefoth et al. 2012 for the full description of the experimental setup). The feedings spots were later also used as angling sites. As argued in Klefoth et al. (2012), the shelter structure was assumed to be the safest habitat within each pond as it provided shelter and no possibility for bird predation events. To reach the feeding spots, the fish had to cross a comparably large open area, similar to a standard open-field test used to measure boldness in laboratory environments with fishes (Budaev 1997). Both shelter and feedings spots were covered by PIT antennae (Fig. 1) enabling the quantification of the individual number of visits at the two feeding spots and the time spent sheltering as three measures of boldness (Klefoth et al. 2012). Low scores of the refuge time and large numbers of visits on feedings spots were assumed to indicate boldness. Functionality of the PIT system was confirmed prior to pond experiments (Appendix 2). Fish were allowed to acclimatize for two days before a behavioral observation period of six days started. During the six day initial personality assessment period, fish were fed daily (5 mm carp pellets, Aller Classic, Aller Aqua, Golßen, Germany) with a total amount of 1% of the pond's population mass (assessed at the release time) from 2 h before sunset until 2 h after sunset on an hourly basis while alternating between the two feeding spots to control for potential impacts of daytime on boldness measurements.

To assess the angling-vulnerability of individual carp carrying specific phenotypes, experimental carp angling was conducted for seven consecutive days after the initial six day observation period. Carp were angled every day for four consecutive hours, and the angling location alternated between the close and the distant feeding spot on an hourly basis. The complete procedure followed the angling protocol described by Klefoth et al. (2013a). Sweet corn was used as bait offered on a standard bolt-rig, which is known to result in 100 % of shallow hooking in the mouth region (Rapp et al. 2008). Materials used for angling followed standard practice in specialized carp angling (Arlinghaus and Mehner 2003). Hooked fish were identified by the PIT tag (Pocket reader, Allflex, Dallas, Texas, USA). Afterwards fish were immediately released back into the pond. This procedure lasted a maximum of 30 s.

The whole experimental procedure within the pond environment lasted 13 consecutive days (six days of behavioral observations without angling, followed by seven days of angling). Mean water temperature ± SD in the ponds over the study period was 21.3 ± 1.0 °C (range: 19.0 – 23.3 °C), and all other environmental variables were within the optimal range for carp. During the study period of experiment 1 two carp disappeared from the ponds likely due to bird predation. The remaining N = 94 carp were used for analyses.

Assessment of personality, morphology, energetic status and vulnerability to longer term angling in ponds (experiment 2)

To further investigate selection pressures on carp phenotypes over a longer fishing period, the pond angling experiment was repeated using a new set of carp (N = 120, mean TL ± SD 199 ± 9 mm) from the same cohort. The second pond experiment followed the same protocol as described for experiment 1 with the exception that angling was conducted on 20 days and additionally growth rate of carp was assessed over a total of 58 days within ponds, which was not possible in experiment 1. No attempt was made to measure personality in the laboratory again, given the low reliability of the assessments in the first trial (Appendix 1). Moreover, we omitted the assessment of the energetic status prior to release into the ponds because of the little predictive power of this variable in relation to angling vulnerability in experiment 1. After the 20 day angling period ended, we continued to feed the fish with 1 % of their initial population body mass per day for another 30 days to determine growth of the fish. The feeding procedure followed the same protocol as described for experiment 1 during undisturbed behavioral observations. Then ponds were drained and fish were again measured for their total length to assess growth increment. Mean water temperature ± SD in the ponds during undisturbed behavioral observations and the first seven days of angling in experiment 2 was 19.0 ± 0.5 °C (range: 17.0 – 20.2 °C), which was similar to experiment 1. Mean water temperature ± SD during angling days 8 - 20 dropped and was 14.9 ± 0.9 °C (range: 13.9 - 17.0 °C). The temperature was 13.3 ± 1.3 °C (range: 11.2 - 16.2 °C) during the subsequent feeding period without angling. After draining the ponds, N = 94 carp provided a full dataset starting with PIT implantation until completed growth measurements. The other 26 individuals presumably disappeared due to predation (N = 11) or lost their PIT tags (N = 15), which is known to be a problem in carp tagging studies (Økland et al. 2003). As indicated by our PIT system data, mortalities and tag loss mainly occurred during the last two weeks of the additional feeding period, and mortalities were randomly distributed between the ponds (either three or four individuals died in each pond). Therefore, food distribution among individuals remained constant over the duration of experiment 2.

Statistical analyses

Personality in the laboratory (experiment 1)

To identify the potential of consistent personality traits measured under standardized environmental conditions in the laboratory all behavioral variables measured in the first four trials using N = 36 carp were included in a principal component analysis (PCA) with varimax rotation (Appendix 1). This was done to identify suites of correlated behaviors that would load strongly on distinct axes, which in turn would be interpretable as distinct personality traits. We repeated the PCA for all behavioral metrics in the second set of trials measured six weeks later. Behavioral measures with a minimum factor loading of 0.5 were considered meaningful for component interpretation (Peres-Neto et al. 2003). Although we failed to identify a stable solution with respect to the principal components capturing an identical set of behavioral metrics, repeatability of behaviors following Lessells and Boag (1987) and rank order consistency over time (Spearman rank correlations) of principal components were nevertheless calculated using results of the first PCA from the first repeat. To that end, we summed z-scores of individual variables shown to load heavily on the same factor in PCA analyses of the first trial, and correspondingly used summed z-scores of the same variables from the second trial to calculate repeatability. Because the initial repeatability analyses provided weak evidence of repeatable behavior, exploration behavior and boldness related behavior for all N = 94 individuals were included in PCA analyses with varimax rotation (Tab. 3). We used data from the resulting two principal components, which explained 50.7 % (PCA 1-boldness) and 25.6 % (PCA 2-exploration) of the variation for further analyses. The resulting PCA-scores were used to test whether laboratory behavior would be predictive of angling vulnerability in ponds and to test for correlations between laboratory behavior and comparable (z-standardized) measurements within the ponds (use of shelter structure and visits at the close and the distant feeding spot) using Pearson`s correlations.

Pond behavior (experiments 1+2)

Using the raw PIT detection data three boldness related measures characterizing individual carp were derived following the protocols described in Klefoth et al. (2012). For each individual fish, the mean “time spent sheltering” per day (expressed as mean minutes h^{-1}) and the mean “number of visits at the feeding spots” per day (expressed as mean # h^{-1}) were estimated, the latter separately for the close and the distant feeding spot. The repeatability of behaviors within ponds was estimated following Lessels and Boag (1987) using mean values from the first week (behavioral observation without angling) and the second week (seven days of angling) for both experiments separately. For subsequent analyses of angling-induced selection on behavior, mean values for each of the three boldness measures per individual fish during the first week of pond behavior undisrupted by angling were estimated. A correlation matrix for all variables included in analyses in both experiments and comprising the correlation of boldness prior to the onset of angling and growth as determined over 58 days in experiment 2 was calculated using Pearson`s correlations.

Morphological traits (experiments 1+2)

The body shape and the shape of the head of each individual were examined as morphological traits potentially correlated with angling vulnerability using a landmark-based assessment approach (Rohlf and Marcus 1993). To that end, we digitized a total of 16 landmarks on the left side of each specimen using the tpsDig2 software (http://life.bio.sunysb.edu/morph) (Fig. 2). The landmarks were: 1) tip of the upper jaw, 2) posterior corner of the upper jaw, 3) corner of the insertion of the pectoral fin, 4) insertion of the pelvic fin, 5) anterior insertion of the anal fin, 6) posterior insertion of the anal fin, 7) ventral point of maximum curvature of the peduncle, 8) posterior extremity of the lateral line, 9) dorsal point of maximum curvature of the peduncle, 10) posterior insertion of the dorsal fin, 11) anterior insertion of the dorsal fin, 12) dorsal insertion of the head, 13) dorsal edge of head perpendicular, 14) center of the eye, 15) ventral edge of head perpendicular, 16) posterior end of operculum (Fig. 2). Raw coordinates were superimposed using general Procrustes superimposition in software MorphoJ 1.03 (Klingenberg 2011). To eliminate potential effects of dorsoventral bending (called arching effect), Burnaby`s orthogonal projection following Valentin et al. (2008) was applied to remove the arching effect. Arching effects were found to be low. Explained variances of the subsequent PCA analyses were reduced by less than 5 % as a consequence of the correction procedure, indicating low bending of the photographed fish. Arching-free shape descriptors were used for subsequent analyses. Principal component analyses (PCA) of Procrustes shape co-ordinates were performed separately using MorphoJ. To further investigate potential impacts of the head morphology on angling vulnerability (Alós et al. 2014b), landmarks 1, 2, 12, 13, 15 and 16 were separately analyzed (Fig. 2). We used data from the resulting first principal components, which explained 34.5 % (full body shape) and 18.0 % (head shape) of the variation in experiment 1 and 13.4 % and 43.6 % in experiment 2, respectively. To control for the effect of size on morphology, residuals of linear regressions between factor scores of the first principal components and total length were calculated and used for further analyses.

Growth (experiments 1+2)

All fish used during the two experiments were raised in the same common garden under natural conditions and were descendants of the same pool of parental fish. Afterwards, all fish experienced the same holdings conditions and the same food levels. Because environmental conditions were equal for all fish prior to experimentation, differences in size between individuals at the onset of experiment 1 reflected differences in growth over the life-span. Thus, size of the fish (TL, mm) was interpreted as a surrogate for growth and used as a predictor variable to calculate fitness in the angling fishery in experiment 1. Further, absolute growth increments (mm) over a 58 day period were calculated for fish in experiment 2. Because fishing may select on growth via behavior (Biro and Sampson 2015), potentially correlated effects of boldness on growth were separated by using residuals of a linear regression between growth increment and boldness in ponds (visits at the distant feeding spot) for further analyses.

Mean standardized selection gradients (β_μ) induced by angling on adaptive traits (experiments 1+2)

In a fishing context, the survival component of fitness is defined by the capture event. Accordingly, a fish was considered theoretically dead (coded as zero) if it was captured in the experimental fishing and otherwise considered alive (coded one). Individual recaptures that occurred during experimental angling were not considered further. We used a nested logistic regression approach considering individual fish nested within replicated ponds to analyze predictors of survival of carp exposed to an angling fishery using boldness-related behaviors, morphology, energetic status and growth (*sensu* length in experiment 1) as predictors. All predictor variables were z-standardized to a mean of 0 and a SD of 1 prior to inclusion into the regression model. A maximum of 9 predictor variables were analyzed to determine fitness of the carp. These variables were: 1) laboratory boldness behavior (BL1) based on PCA 1 scores (only in experiment 1), 2) laboratory exploration behavior (BL2) based on PCA 2 scores (only in experiment 1), 3) body fat content at the time of stocking (Fat) (only in experiment 1), 4) total length at the time of stocking within ponds (TL), 5) body shape (SB) and 6) head shape (SH), both based on our morphological analyses, 7) number of visits at the close and the distant feeding spot within ponds as an indicator of boldness under semi-natural conditions in groups (BP), 8) time spent sheltering within ponds as a further measure of boldness in ponds (SP), and 9) growth rate in ponds over 58 days (G) (only in experiment 2). In case of the "BP" variable, only the distant feeding spot was ultimately considered in the final models. This was done because the number of visits at the close and the distant feeding spot were moderately to highly correlated (Pearson`s correlation between the close and the distant feeding spot in experiments 1 and 2 $r = 0.337$, $P = 0.001$ and $r = 0.887$, $P < 0.001$, respectively) and the distant feeding spot was assumed to have been perceived as particularly risky by the fish. Consequently it was assumed to constitute the most robust measure of boldness (Klefoth et al. 2012). Our starting models for experiments 1 and 2 were:

$$\mathrm{logit}(s)_{(\text{experiment 1})} = \alpha_0 + \alpha_1 * BL1 + \alpha_2 * BL2 + \alpha_3 * BP + \alpha_4 * TL + \alpha_5 * Fat + \alpha_6 * S + \alpha_7 * SH + \alpha_8 * SP + \alpha_9 * TL^2 + \alpha_{10} * BP^2 \text{ and}$$

$$\mathrm{logit}(s)_{(\text{experiment 2})} = \alpha_0 + \alpha_1 * BP + \alpha_2 * TL + \alpha_3 * G + \alpha_4 * S + \alpha_5 * SH + \alpha_6 * SP + \alpha_7 * G^2 + \alpha_8 * BP^2$$

For experiment 2, two different models with the same independent variables were calculated, as fitness (i.e., survival of an angling fishery) was based on either short (7 days) or long (20 days) angling durations. All models for both datasets also contained quadratic terms for boldness in ponds and for a measure of growth ("TL" in experiment 1 and "G" in experiment 2) to test for stabilizing or disruptive selection on these traits (Olsen and Moland 2011). The most parsimonious models were selected based on Akaike`s Information Criterion corrected for small sample sizes AIC_c (Burnham et al. 2011). We compared the AIC_c scores between a restricted set of models based on their relevance to explain carp fitness in our experiments rather than testing all possible combinations of predictor variables (Burnham and Anderson 1998, see also Olsen et al. 2012 for a similar approach in a comparable field study). For the best models, the total amount of explained variances was calculated using Nagelkerke`s pseudo R^2.

Multivariate regression models on relative fitness or fitness components such as survival allow the interpretation of regression coefficients as selection gradients following the landmark work by Arnold and Wade (1984). We estimated mean standardizes selection gradients (β_μ) based on logistic regression coefficients to allow comparisons of selection strengths caused by angling among traits carrying different units following the methods described in Matsumura et al. (2012). To that end, logistic regression coefficients for all adaptive traits from the final models were transformed to their linear equivalents following Janzen and Stern (1998). The resulting unstandardized selection gradients represented the SD-standardized selection gradients because traits were initially standardized to a mean of zero and a SD of 1 (Matsumura et al. 2012). To estimate β_μ as unitless measures of strength of selection, selection gradients were multiplied by the original mean and divided by the original SD of the phenotypic trait (Matsumura et al. 2012). The β_μ is preferred for representing selection in the wild and it represents the relative change in fitness that results from doubling of the trait value (Matsumura et al. 2012). The measure allows comparisons of the strength of selection acting on several traits that differ in units, means and variance (Hereford et al. 2004, Matsumura et al. 2012).

Logistic regression analyses were conducted using the software package R version 3.1.2 (R Development Core Team) by applying the library lme4 (Bates et al. 2014), and AIC_c values were calculated using library AICcmodavg (Mazerolle 2013). Principal component analyses and all Pearson and Spearman rank correlations applied in this study were conducted using software package SPSS 20. All authors consent the research presented in this article.

Results

Personality of individual carp assessed individually in the laboratory

Principal component analyses of all four behavioral contexts (exploration, boldness, novel object, shoaling) using N = 36 fish revealed two principal components explaining 46.0 % (based on variables from the boldness and the novel object context, interpreted as boldness; PCA 1) and 22.2 % (based on variables from the exploration and the shoaling context, interpreted as exploration; PCA 2) of the total variance at the first trial, and 35.3 % (based on variables from the exploration and the boldness context; PCA 1) and 24.5 % (based on the exploration, novel object and the shoaling context; PCA 2) of the total variance at the second trial (Appendix 1). However, the variables loading on these two PCA`s differed substantially between the first and the second measurement six weeks later, indicating low consistency of observed behaviors within the laboratory (Appendix 1). The corresponding repeatability analyses of the summed z-scores of all behaviors loading on the first and the second PCA of the first trial, re-tested six weeks apart indicated no significant repeatable behavior between the first and the second trial (Tab. 2). However, the variables loading heavily on the first PCA describing risk taking behaviors in the context of foraging (i.e., boldness) were found to be more repeatable than exploration behaviors and in fact repeatability scores were close to significant with just N = 36 fish (Table 2, Appendix 1). Hence, we applied the boldness and the exploration trials and used the resulting data from individually assayed carp to determine angling vulnerability in ponds.

Principal component analyses of the exploration and the boldness context including data of all N = 94 carp from experiment 1 again revealed two PCA`s, explaining 50.7 % and

25.6 % of the total variance, respectively. PCA 1 included all behavioral measures taken from the boldness context containing the "Time to leave the refuge", the "Time to ingest the 1st food item" and the "Time to ingest the 2nd food item after disturbance" and PCA 2 included only one measure - the "Time to leave the refuge" within the exploration context (Tab. 3, Appendix 1). The time spent within the upper water zone showed factor scores below 0.5 and was therefore not considered anymore. We interpreted the two PCA axes as boldness and exploration behavior as revealed also for the smaller data set mentioned before. PCA scores from PCA 1 and PCA 2 were weakly but not significantly related to the time spent sheltering within ponds (Pearson`s $r = 0.179$ and 0.178, $P = 0.084$ and 0.086, respectively, Appendix 1), and both factors were unrelated to the visits at the two feeding spots within ponds (all Pearson`s $r < 0.13$, all $P > 0.05$; Tab. 1, Appendix 1). These findings showed that the behavior of individual carp in the laboratory environment did not correspond with boldness-related behaviors expressed in groups in the ponds.

Personality of individual carp assessed in groups in the pond

In contrast to the lack of strong personality shown by carp in the laboratory (Appendix 1), boldness-related carp behavior in the ponds assessed in groups was found to be highly consistent and repeatable, indicating personality with respect to boldness. Repeatability estimates for all boldness measures (visits of feeding spots and use of the shelter) were very high and significant ranging between $r = 0.63$ and $r = 0.70$ in experiment 1 and between $r = 0.53$ and $r = 0.74$ in experiment 2 with significant underlying F-statistics and Spearman correlations in all cases (Tab. 4).

Angling vulnerability

During the seven day fishing period in experiment 1, a total of 23 individual carp out of 94 (25 % of the total population, 24.6 ± 5.6 % per pond, $N = 3$) was captured in 84 rod-angling hours. In experiment 2, 38 carp out of 94 individuals were captured during the first 7 days of angling (40 % of the total population, 40.1 ± 6.3 % per pond, $N = 3$) within 84 rod-angling hours, and over 20 angling days in experiment 2 with 240 rod-angling hours a total amount of 49 carp were captured (53 % of the total population, 51.8 ± 6.1 % per pond, $N = 3$). Catch per unit effort (CPUE) was 0.28 fish h^{-1} in experiment 1, 0.46 fish h^{-1} during the first seven days of experiment 2 and 0.21 fish h^{-1} over the complete course of experiment 2.

The captured individuals in both experiments were on average larger, grew faster and behaved bolder compared to their uncaught conspecifics (Table 5). Logistic regression analyses and AIC_c-based model selection in experiment 1 revealed larger fish (TL), bold behavior in ponds (BP), bold behavior within the laboratory in the context of foraging (BL1) and body shape (SB) with slightly deeper bodies (compare Fig. 4) to be positively related to angling vulnerability (Tab. 6, 7; Fig. 3, 4). However, at a ΔAIC_c of only 0.2, an equally supported model excluded the laboratory behavior (BL1), indicating laboratory behavior to relate only little to angling vulnerability (Tab. 6). Exploration behavior as measured in the laboratory or the ponds (BL2 and SP) was never retained in the best models. Similarly, the energetic status (Fat) was not included in the best model in experiment 1. In the first seven days of angling in experiment 2, the best model explaining survival-based fitness of carp in our angling fishery consisted of size (TL), growth (G) and boldness within ponds (BP) (Tab. 6, 7; Fig. 3). Analyzing twenty days of angling revealed three models within a narrow ΔAIC_c range of 0.8, which included boldness within ponds (BP), growth (G), body shape (SB) and size of the head and mouth (SH) to best explain fitness in our carp fishery (Tab. 6, 7; Fig. 3, 4). Size of the fish (TL) was no longer present in the best supported model.

Mean standardized selection gradients allowed direct comparisons of the strength and direction of angling-induced selection acting on each of the traits included in the best supported survival models. During the short-term angling fishery (7 days) in both experiments the size of the carp (with large fish being more likely to be captured) was more than seven times more strongly under selection than boldness-related behavioral traits within ponds, with bold fish being more likely to be captured than shy individuals (Tab. 5, 7). In the short-term angling fishery in experiment 2, the morphological variable TL also exerted much greater influence on vulnerability than juvenile growth rate (G) (Tab. 7). However, over the longer fishing period of 20 angling days in experiment 2, highest normalized selection gradients were acting on the boldness of the fish (BP) - a value which was 1.5 times greater than the selection acting directly on growth (G). Here, TL of the fish no longer explained survival of carp in the angling fishery (Tab. 6). Correlation analysis revealed the growth (G) of the fish in experiment 2 to be only moderately correlated with the number of visits at the distant feeding spot as a measure of pond boldness (BP) (Pearson`s $r = 0.310$, $P = 0.002$; Tab. 1). Overall, within our size-restricted set of experimental fish, boldness-related behavior was found to be the most important trait under selection by angling over a period of 20 days, whereas size (TL) and growth (G) had lower (as observed for G) or no (as observed for TL) importance for determining vulnerability to angling when fishing took place over a 20 days angling period (Tab. 6, 7). Hence, angling selection acted directly and most strongly on resource acquisition-related behavior and only secondarily on juvenile growth rate. Only negligible selection pressures were found to act on laboratory behavior (BL1), body shape (SB) and size of the head and mouth (SH) (Tab. 7; Fig. 4), and a lower fitness was revealed for more deeply bodied fish and for carp with larger heads. We found no evidence for energetic status (Fat) to determine angling vulnerability. There was also no sign of disruptive selection as no quadratic terms were retained in the best supported models.

Discussion

Our study provided strong support for the hypothesis that a passive fishery with hook and line directly selects on behavioral traits related to risk-taking during foraging (i.e., boldness) as expressed by angling-naïve groups of carp in semi-natural replicated ponds. In fact, we found the standardized selection pressure on boldness to be much stronger than angling-induced selection acting directly on juvenile growth rate so that one might expect a largely independent selection response to angling in boldness without a corresponding change in juvenile growth rate. In contrast to recent laboratory data presented on trapping-induced selection on crayfish by Biro and Sampson (2015), we only found a modest correlation between boldness and growth. However, also this modest correlation might induce an indirect selection gradient on juvenile growth rate and might lead to a correlated selection response as previously argued by Biro and Post (2008) and Uusi-Heikkilä et al. (2008). Hence, a potentially observed selection response in (juvenile) growth rate in phenotypic data in the wild (e.g., Edeline et al. 2007, Swain et al. 2007) may well be mechanistically caused as an indirect response to selection on resource acquisition-related behaviors such as boldness or activity. Our study joins other recent work emphasizing the importance of behavior in the context of fishing-induced selection (Wilson et al. 2011, Olsen et al. 2012, Alós et al. 2012, 2015, Härkönen et al. 2014, 2015, Biro and Sampson 2015) but is novel insofar as it reveals the relative importance of behavioral selection in comparison to other morphological and life-history traits.

We showed that boldness in ponds is a dominant trait under selection in passive angling fisheries, which suggests that over time exploited populations of fishes should be increasingly timid as observed in field studies of intensively exploited coastal fishes (Alós et al. 2015). However, this pattern only emerged in an angling period of 20 days. Initially, in both of our seven day fishing period (experiment 1+2), the selection pressures acting on total length (a surrogate for life-time growth) was stronger than the strength of selection acting on boldness. As time progressed, increasing numbers of smaller, yet very bold individuals that visited the feedings spots repeatedly were hooked, "washing" down the selection pressure on length and growth rate until length dropped from the best predictive models and boldness became dominant. However, even after accounting for bold behavior, some selection continued to act on growth rate expressed in the ponds. It is very likely that fish with high growth-rates not only visited the feeding spots more often, but also consumed more particles as previously documented for bold domesticated carp in comparison with shyer wild-like conspecifics (Klefoth et al. 2013a). Results from piscivorous largemouth bass, selected for their individual vulnerability confirm this assumption as highly vulnerable fish were shown to have higher attack rates and lower prey rejection rates (Nannini et al. 2011). Growth rate also likely integrated the independent effects of some other physiological and behavioral traits. Hence, due to the sometimes observed link between boldness, learning abilities (DePasquale et al. 2014, Kotrschal et al. 2014, Trompf and Brown 2014) and metabolism (Biro and Stamps 2010) selective fishing has the potential to influence a wide range of correlated phenotypic expressions (Uusi-Heikkilä et al. 2008). In fact, it is difficult to think about a non-behavioral mechanism explaining the residual selection operating on juvenile growth rate other than a correlation among metabolism and growth rate (Biro and Stamps 2010), which might render the faster growing fish to have greater hunger levels. In line with Biro and Sampson (2015) we thus tentatively conclude that a sizable fraction of the remaining "direct" selection on juvenile growth rate can be explained by some energy-acquisition related behaviors (Enberg et al. 2012) that were not measured in our study.

The lack of selection on size in a longer-term, as observed in our study should not be over-interpreted because we purposely used fish of a very narrow size range to maximize behavioral variation and to control for the undisputed importance of size for vulnerability. Larger fish under natural conditions generally show higher swimming speeds (Stamps 2007), have larger gape sizes, are often dominant (Jenkins 1969), often have larger home ranges (Nash et al. 2015) and are characterized by larger consumptive demands compared to smaller fish (Clarke and Johnston 1999, Mittelbach et al. 2014), likely leading to intrinsically larger vulnerability to passive angling gear in large compared to small individuals. Indeed, Beukema and De Vos (1974) observed larger-than-average carp from two replicated ponds to be 20-30 % more likely to be captured by angling than their smaller-than-average counterparts from the same water bodies. One would thus expect selection on size to be present under natural conditions.

Boldness is defined as an individual`s reaction to any risky, but not novel situation (Réale et al. 2007), which was found to directly relate to angling vulnerability in the current and a previous study on carp (Klefoth et al. 2013a). Further, we found the pond behavior assessed in groups, but not the individually assayed laboratory behavior of carp to be highly repeatable and subsequently related to angling vulnerability, which agrees with previous work on harvesting-induced selection on behaviors in Atlantic cod (*Gadus morhua*) (Olsen et al. 2012). Our results also confirm recent studies that implied relationships among behavioral traits (e.g., exploration, Alós et al. 2012, Härkönen et al. 2014, 2015, or aggression, Redpath 2009, 2010, Sutter et al. 2012) and vulnerability to angling. However, other studies failed to relate measures of boldness and vulnerability to angling in centrachids (Wilson et al. 2011) and percids (Kekäläinen et al. 2014, Vainikka et al. in press), either indicating a species-specific response or methodological artefacts. Indeed, all studies that failed to relate boldness related behaviors to angling vulnerability were conducted in laboratory environments (Wilson et al. 2011, Kekäläinen et al. 2014, Vainikka et al. in press) and angling vulnerability was measured in small laboratory tanks where all personality types could easily access the offered bait (as in Wilson et al. 2011). There is increasing evidence that boldness assessments within a benign laboratory are less repeatable compared to studies under more natural conditions and with an explicit predation threat involved (Bell et al. 2009, Toms et al. 2010, Klefoth et al. 2012). Because consistent and repeatable individual variation of behavior is a prerequisite when aiming to show a selection response, rapid assaying of behavior in the laboratory might be unsuitable as this can introduce serious bias in personality assessments (Biro 2012) and can lead to misinterpretation of its ecological and evolutionary consequences (Niemelä and Dingemanse 2014). We therefore contend that despite the obvious benefits of controlled individual behavioral phenotyping in the laboratory, field experiments are of paramount importance to understand the mechanistic basis of angling vulnerability.

The negative selection gradients estimated on juvenile growth rate in the present study on first sight seem to support the "intuition" (Walters and Martell 2004) that heavily exploited carp (and ecologically similar species such as bream, *Abramis brama*, or tench, *Tinca tinca*) stocks should host individuals that grow less when adult, in line with empirical evidence in salmonids (Saura et al. 2010), esocids (Edeline et al. 2007) and several coastal and marine fishes (Swain et al. 2007, Alós et al. 2014a). However, this does not mean that evolution of small growth rate is a default response to intensive harvesting, because we found independent selection gradients acting on boldness and juvenile growth rate in carp. Hence, an evolutionary response of just boldness, just growth rate or both is possible depending on the local fitness landscape and the degree to which natural selection works in opposite directions to fishing selection (Edeline et al. 2007). Only species- and fishery-specific analysis that account for the life-time fitness of specific trait values can provide conclusive answers (Laugen et al. 2014). Moreover, the natural fitness benefits of fast growth and large size might easily overrule any angling-induced negative selection gradients acting directly or indirectly on juvenile growth rate (Matsumura et al. 2011). Hence, depending on the species, fisheries-induced selection of either fast, slow or no change in juvenile growth rate can happen (Dunlop et al. 2009, Matsumura et al. 2011, Enberg et al. 2012).

Our experiments showed that bolder and faster growing carp were more easily captured than shyer and less fast growing conspecifics. Whereas vulnerability to angling gear in piscivorous largemouth bass is mainly driven by aggression towards artificial lures (Philipp et al. 2009, Sutter et al. 2012), we could show that the vulnerability of omnivorous carp is based on behaviors related to energy acquisition of sessile prey. The prerequisite for growth is energy intake and under situations of food limitation and non-random food distribution, as it is typical for natural ecosystems, bold individuals energetically benefit from exploring and accessing more resources at the costs of exposing themselves to predation-risk. Shy individuals, i.e., those individuals positively selected under fished conditions, are characterized by a strongly risk-sensitive life-style (Réale et al. 2010). Irrespective of this, shy individuals might still have sufficient access to resources because they might utilize the safe periods to ingest food, which is easily possible also in bottom-feeding cyprinds that forage on sessile prey. Hence, fisheries-induced evolution towards increased shyness could in theory also maintain genetically fast growing individuals. However, largemouth bass selected over three generations for high vulnerability grew less in the juvenile life stage in likely food limited ponds compared to bass of low vulnerability to angling (Redpath et al. 2009, 2010). This outcome was likely caused by the positive correlation of metabolic rate and vulnerability, which carries energetic costs to high vulnerability phenotypes. Whereas selection on aggression through correlated selection responses of metabolic rate, as observed in largemouth bass, will usually lead to reductions in realized growth (Redpath et al. 2010), cyprinids like carp will either maintain growth potential or evolve smaller growth in response to selection on boldness. Piscivorous fish like largemouth bass that are selected based on aggression might develop faster realized juvenile growth due to reductions in standard metabolism associated with the selective advantage of low aggressive phenotypes. Conclusively determining the direction of change in life-histories in response to selection on behavior will thus depend on the species foraging ecology and physiological underpinnings of trait expression. Much more work is needed to answers these highly relevant questions for intensively exploited stocks.

We found no relationship between the energetic status of the fish, as measured by relative body fat content, and their vulnerability to angling. Our measurement technique using energy meters to non-lethally detect relative body water- and body fat contents has been proven to be a reliable tool (Crossin and Hinch 2005, Klefoth et al. 2013b), and it was assumed that the energetic status of the fish potentially influences the vulnerability to angling (Huse et al. 2000) by being indicative of the short term hunger status of fish (Krause 1993, Nakayama et al. 2012). However, based our findings, energetic status was likely not a valid measure of hunger level. It is difficult to imagine that hunger does not influence catchability, and in fact previous work has shown that under conditions where natural food becomes scarce fish become very vulnerable to harvest (Raat 1991, Herrman et al. 2013). Possibly, hunger was better reflected by our measures of pond boldness and was completely unrelated to the energy status measured in the somatic tissue at release. It is also conceivable that the energetic status of fish changed quickly in the ponds after release, leading to a situation where the measurement of relative body fat content at the time of stocking did not properly reflect the energetic status of the fish at the time of capture.

The body shape of the fish as determined by geometric morphometrics only added little to the suite of phenotypes under selection in our angling fishery. Whereas Alós et al. (2014b) found comparatively strong evidence for angling-induced selection on large mouth size and streamlined bodies after controlling for size, condition and fishing site in heavily exploited Mediterranean coastal fishes, we could only detect little effects of body shape and head size and mouth on an individual`s fitness in a passive hook and line fishery. Direct physical interactions of the mouth with the fishing gear can explain why individuals with a larger mouth are more likely to be captured (Alós et al. 2014b) as an increasing gape size facilities ingestion of the hook (Alós et al. 2008). Similarly, in our carp, we found weak evidence that larger heads and mouths positively influenced vulnerability of the fish. In contrast to Alós et al. (2014b), however, we found some evidence of deeply-bodied fish to be more likely to be selected compared to more streamlined individuals. Deep bodies are indicative of domestication selection in carp, and it was previously reported at the group level that more domesticated carp are on average more vulnerable to angling than less domesticated individuals because the domesticated ones take more risks and feed more (Beukema 1969, Huntingford 2004, Klefoth et al. 2012, 2013a). In addition, our results indicate the strongest selection to act on bold behavior, and therefore correlated traits like body shape and head size might simply act as a surrogate for behavior (Alós et al. 2014b). In turn, selection on morphological properties might appear stronger in the absence of direct measures of behavior as in the case of Alós et al. (2014b).

In conclusion, our study is the first in fishes to mechanistically show that selection on juvenile growth rate can happen as an indirect response to direct selection on behavior and that behavioral traits might be under very strong selection in passively operated angling fisheries as it was recently shown for crayfish (Biro and Sampson 2015). We further found support for the productivity-personality hypothesis (Stamps 2007, Biro and Stamps 2008), which predicts that boldness-related behavior can be directly linked to resource acquisition and growth in omnivorous carp. The ultimate direction of the evolutionary response will depend on the heritability of the selected traits and on the relative strength of simultaneously acting natural and harvest selection (Edeline et al. 2007). Under natural conditions in repeat spawners, large body size often maximizes life-time fitness (Roff 1984, Olsen and Moland 2011, Alós et al. 2014a), but there is an optimal growth rate to be expected given the unavoidable trade off of growth and mortality (Stamps 2007). Because in omnivorous fishes like carp fast growth of early life stages should be favored to outgrow gape size limited predators and to maximize body size at first reproduction, the ultimate selection response to positively size-selective harvest will be weakened by natural selection working in the opposite direction (Edeline et al. 2007). However, we found boldness to be under strongest selection in our passive fishery and only a modest correlation of boldness and growth rate [in contrast to the crayfish in Biro and Sampson (2015)]. Boldness may be less directly linked to life-time reproductive fitness compared to size and growth and indeed, the heritability of boldness and other behaviors has been found to be substantially greater compared to life-history traits like growth (Mousseau and Roff 1987). Coupled with the strong selection gradients acting on boldness, we predict that the evolutionary response of boldness-related behaviors in response to recreational harvesting should be strong. As a consequence, intensive angling fisheries should leave behind individuals that are more timid and harder to catch (Philipp et al. 2009, Tsuboi et al. in press), a pattern that might be further reinforced by learning to avoid future capture (Klefoth et al. 2013a, Philipp et al. 2015). The increased levels of timidity might ultimately affect entire food webs through trait-mediated indirect interactions (Werner and Peacor 2003) in a "landscape of fear" (Laundré et al. 2014). Further, because we found laboratory assessments of carp personality to be highly unreliable, we encourage more field work in studies on fishing-induced selection and evolution that also contain a behavioral perspective of the fish (and potentially also of anglers, Matthias et al. 2014) and that focus on several important phenotypic traits at the same time to further disentangle suits of characters potentially under selection in fisheries.

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Tables

Tab. 1: Correlation matrix of z-standardized variables involved in experiment 1 (a) and experiment 2 (b). BL1 = laboratory boldness behavior, BL2 = laboratory exploration behavior, Fat = body fat content at the time of stocking, TL = total length at the time of stocking within ponds, SB = body shape, SH = head shape, BP = number of visits at the distant feeding spot within ponds, SP = time spent sheltering within ponds, G = growth rate in ponds over 58 days.

(a)

Trait	BL1	BL2	BP	TL	Fat	SB	SH	SP
BL1	1	0	-0.104	0.150	-0.097	0.126	0.069	0.179
BL2		1	-0.128	-0.299	0.006	-0.127	-0.170	0.178
BP			1	0.065	0.108	-0.121	0.076	-0.099
TL				1	-0.100	0	0	0.164
Fat					1	-0.010	-0.071	0.056
SB						1	0.307	0.191
SH							1	-0.012
SP								1

(b)

Trait	BP	TL	G	SB	SH	SP
BP	1	0.100	0.310	-0.248	-0.148	-0.521
TL		1	0.047	0	0	-0.024
G			1	-0.129	-0.133	-0.191
SB				1	-0.164	0.090
SH					1	0.037
SP						1

Tab. 2: Rank order consistency and repeatability of summed z-scores as classified by PCA analyses with varimax rotation based on boldness behaviors (PCA 1) and exploration behavior (PCA 2) in four different contexts based on N = 36 juvenile carp individually assayed under laboratory conditions (see Appendix 1 for details).

	Rank order consistency			Repeatability		
PCA 1	$r_s = 0.312$	$P = 0.064$	$N = 36$	$F = 1.694$	$P = 0.060$	$r = 0.256$
PCA 2	$r_s = 0.204$	$P = 0.233$	$N = 36$	$F = 1.354$	$P = 0.185$	$r = 0.150$

Tab. 3: Component loadings of PCA analysis based on behavioral measures within two different contexts (exploration and boldness) based on all N = 94 fish in experiment 1.

Behavioral trait (experiment 1, Laboratory)	Principal Components	
	PCA 1 (BL1) Boldness	PCA 2 (BL2) Exploration
Time to leave the refuge in exploration trial		0.981
Time to leave the refuge in presumed boldness trial	0.765	
Time to ingest 1st food item in presumed boldness trial	0.896	
Time to ingest 2nd food item in presumed boldness trial	0.793	
Eigenvalue	2.03	1.03
Variance explained %	50.7	25.6

Tab. 4: Rank order consistency and repeatability of boldness-related measures within the pond environment from experiment 1 (N = 94) and experiment 2 (N = 94).

	Rank order consistency				Repeatability		
Variable	Experiment	N	Spearman r	P	F	P	r
Close feeding spot	1	94	0.763	< 0.001	3.090	< 0.001	0.69
Distant feeding spot	1	94	0.676	< 0.001	2.676	< 0.001	0.63
Shelter use	1	94	0.744	< 0.001	3.249	< 0.001	0.70
Close feeding spot	2	94	0.789	< 0.001	2.322	< 0.001	0.58
Distant feeding spot	2	94	0.746	< 0.001	2.101	< 0.001	0.53
Shelter use	2	94	0.647	< 0,001	3.673	< 0.001	0.74

Tab. 5: Mean ± SD values of different behavioral data, total length, body fat content and growth for caught and uncaught individuals in a passive angling fishery from experiment 1 (7 days angling) and experiment 2 (7 days and 20 days angling).

Trait	Captured Mean ± SD	Not Captured Mean ± SD
Experiment 1 (7 d angling)		
Time to leave the refuge (Exploration) (min)	34.5 ± 28.6	23.0 ± 25.5
Time to leave the refuge (Boldness) (min)	20.4 ± 24.6	23.3 ± 24.6
Time to ingest 1st food item (Boldness) (min)	5.3 ± 4.7	7.3 ± 4.0
Time to ingest 2nd food item after disturbance (Boldness) (min)	8.1 ± 3.5	9.1 ± 2.6
Time spent sheltering (pond; min h^{-1})	2.8 ± 2.6	3.1 ± 2.6
Number of visits at the close feeding spot (pond; # h^{-1})	8.6 ± 4.3	6.9 ± 3.4
Number of visits at the distant feeding spot (pond; # h^{-1})	5.5 ± 2.7	4.5 ±2.4
Total length (mm)	185.5 ± 21.3	174.3 ± 15.6
Body fat (%)	5.8 ± 0.7	6.0 ± 0.9
Experiment 2 (7 d angling)		
Time spent sheltering (min h^{-1})	5.7 ± 2.2	6.7 ± 2.8
Number of visits at the close feeding spot (# h^{-1})	5.3 ± 1.1	4.3 ± 1.6
Number of visits at the distant feeding spot (# h^{-1})	5.0 ± 1.2	4.3 ± 1.6
Total length (mm)	201.6 ± 10.0	198.0 ± 8.4
Growth (mm)	9.3 ± 5.1	6.8 ± 5.4
Experiment 2 (20 d angling)		
Time spent sheltering (min h^{-1})	6.0 ± 6.6	6.6 ± 2.5
Number of visits at the close feeding spot (# h^{-1})	5.3 ± 1.3	4.2 ± 1.6
Number of visits at the distant feeding spot (# h^{-1})	5.0 ± 1.4	4.0 ± 1.5
Total length (mm)	200.1 ± 10.7	198.8 ± 7.2
Growth (mm)	9.7 ± 5.3	5.8 ± 4.8

Tab. 6: Logistic regression of carp survival in ponds in experiment 1 (7 d angling) and experiment 2 (7 d and 20 d angling) showing the model structure, number of parameters (#P) and AIC_c values. Bold values indicate models with the lowest AIC_c and a $\Delta AIC_c < 1$. Explanatory variables include BL1 = laboratory boldness behavior, BL2 = laboratory exploration behavior, Fat = body fat content at stocking, TL = total length at stocking, SB = body shape, SH = head shape, BP = number of visits at the distant feeding spot within ponds, SP = time spent sheltering within ponds, G = growth rate in ponds over 58 days.

Model no.	Model structure	#P	AIC_c
Experiment 1 (7 d)			
1	$BL1+BL2+BP+TL+Fat+SB+SH+SP+TL^2+BP^2$	11	114.9
2	$BL1+BL2+BP+TL+Fat+SB+SH+SP+TL^2$	10	112.6
3	BL1+BL2+BP+TL+Fat+SB+SH+SP	9	111.5
4	BL1+BP+TL+Fat+SB+SH+SP	8	109.2
5	BL1+BP+TL+SB+SH+SP	7	107.0
6	BL1+BP+TL+SB+SH	6	104.7
7	BL1+BP+TL+SB	5	**102.4**
8	BP+TL+SB	4	**102.6**
9	BP+TL	3	103.6
10	TL	2	104.0
11	NULL	1	108.7
Experiment 2 (7 d)			
1	$BP+TL+ G+SB+SH+SP+BP^2+G^2$	9	130.1
2	$BP+TL+SB+SH+G+SP+G^2$	8	130.4
3	BP+TL+SB+SH+SP+G	7	129.6
4	BP+TL+SB+SH+G	6	128.0
5	BP+TL+SB+G	5	128.3
6	BP+TL+G	4	**126.3**
7	TL+G	3	129.2
8	G	2	130.5
9	NULL	1	131.0
Experiment 2 (20 d)			
1	$BP+TL+SB+SH+G+SP+BP^2+G^2$	9	130.0
2	$BP+TL+SB+SH+G+SP+G^2$	8	127.4
3	BP+TL+SB+SH+G+SP	7	125.6
4	BP+TL+SB+SH+G	6	123.8
5	BP+SB+SH+G	5	**121.6**
6	BP+SB+G	4	**121.2**
7	BP+G	3	**122.0**
8	G	2	131.3
9	NULL	1	136.4

Tab. 7: Angling-induced selection acting on carp behavior, morphology, growth and energetic state in experiment 1 and 2 showing logistic regression coefficients (α), standard errors (SE), P values (P), mean standardized selection gradients (β_μ) and pseudo R² values. The best models containing the most variables within a $\Delta AIC_c < 1$ in relation to the best models in bold in Tab. 6 are presented.

Variable	α	SE	P	β_μ	R²
Experimental series 1 (7 d angling)					0.18
Laboratory Behavior (BL1)	-0.417	0.26	0.114	$-8{,}37\ 10^{-18}$	
Pond Behavior (BP)	-0.400	0.25	0.113	-0.15	
Total Length (TL)	-0.607	0.28	0.028	-1.23	
Body Shape (S)	-0.440	0.27	0.110	$-3.06\ 10^{-6}$	
Experimental series 2 (7 d angling)					0.17
Pond Behavior (BP)	-0.518	0.24	0.029	-0.437	
Total Length (TL)	-0.373	0.23	0.105	-3.422	
Growth (G)	-0.357	0.23	0.117	-0.288	
Experimental series 2 (20 d angling)					0.30
Pond Behavior (BP)	-0.768	0.24	0.004	-0.655	
Body Shape (S)	0.343	0.25	0.169	$-0.08\ 10^{-6}$	
Head Shape (SH)	-0.340	0.25	0.168	$-9.77\ 10^{-7}$	
Growth (G)	-0.699	0.26	0.007	-0.424	

Figures

Fig. 1: Experimental setup for behavioral observations under laboratory conditions (top) in experiment 1 and under semi-natural pond conditions (bottom) in experiment 1 and 2. Individual boldness assessments under laboratory conditions for all final N = 94 juvenile carp in experiment 1 included an exploration trial where the sliding door was opened and the time until the fish left the refuge was measured, and a boldness trial where in addition to exploration behavior the time until the fish fed the first pellet was measured. Directly after ingestion of the first pellet, a second food item was released together with a fright stimulus (fishing weight) in the boldness trial. Then the time until the second food item was eaten after disturbance was assessed as a further measure of boldness. Within the ponds, boldness was defined in a group setting by low sheltering times and high number of visits at the close and the distant feeding spot (circles). All structures within the ponds were covered by passive integrated transponder antennae (PIT).

Fig. 2: Body shape landmarks (N = 16) used for morphometric analysis in carp.

Fig. 3: Box-plots comparing z-standardized trait values between vulnerable (fitness = 0) and invulnerable (fitness = 1) carp identified in regression models to be under selection in a 7 days lasting passive angling fishery during experiment 1 (left), and experiment 2 (middle), and in a 20 days lasting passive angling fishery in experiment 2 (right). Boxes define the 25th and 75th percentiles and median values are indicated by dark black bars within the boxes.

Fig. 4: Comparison of mean body shape of vulnerable (black line) and invulnerable (grey line) carp in a passive angling fishery in experiment 1 (7 d of angling) and experiment 2 (20 d of angling).

Fig. 1

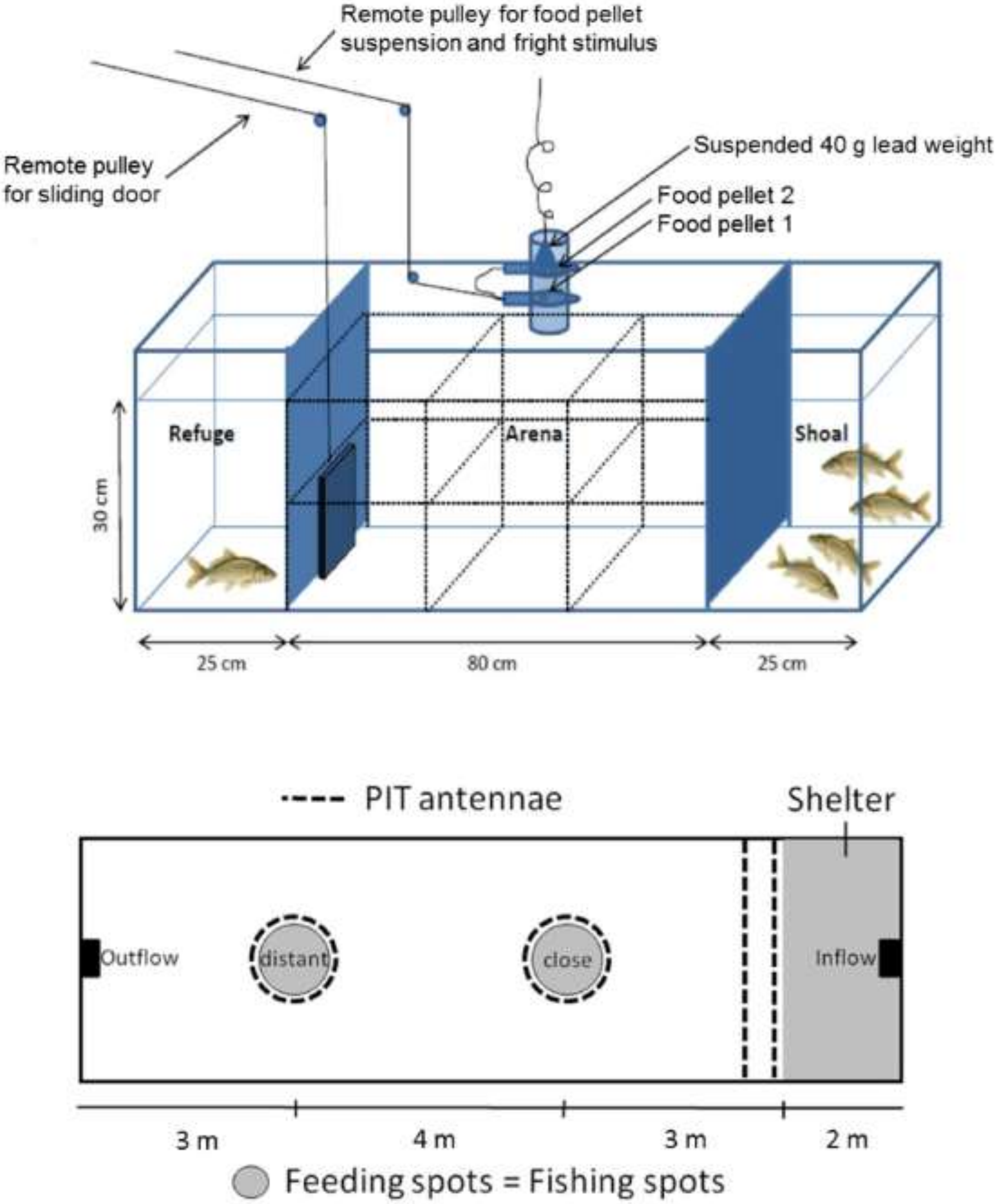

Remote pulley for food pellet suspension and fright stimulus
Remote pulley for sliding door
Suspended 40 g lead weight
Food pellet 2
Food pellet 1
Refuge
Arena
Shoal
30 cm
25 cm
80 cm
25 cm
PIT antennae
Shelter
Outflow
distant
close
Inflow
3 m
4 m
3 m
2 m
Feeding spots = Fishing spots

Fig. 2

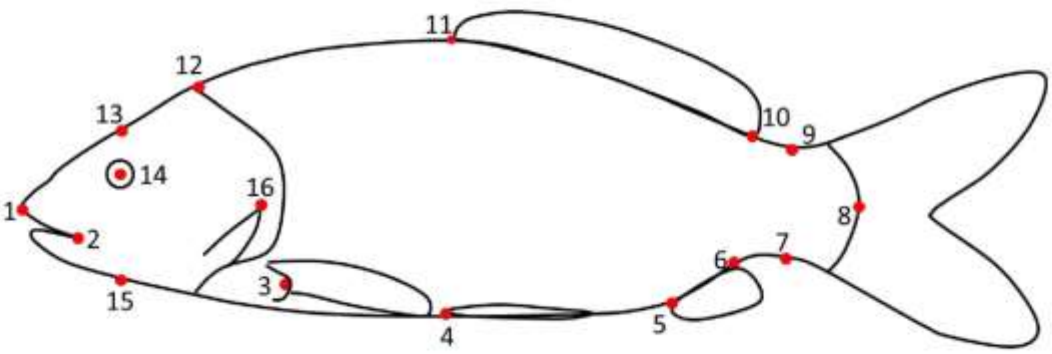


Fig. 3

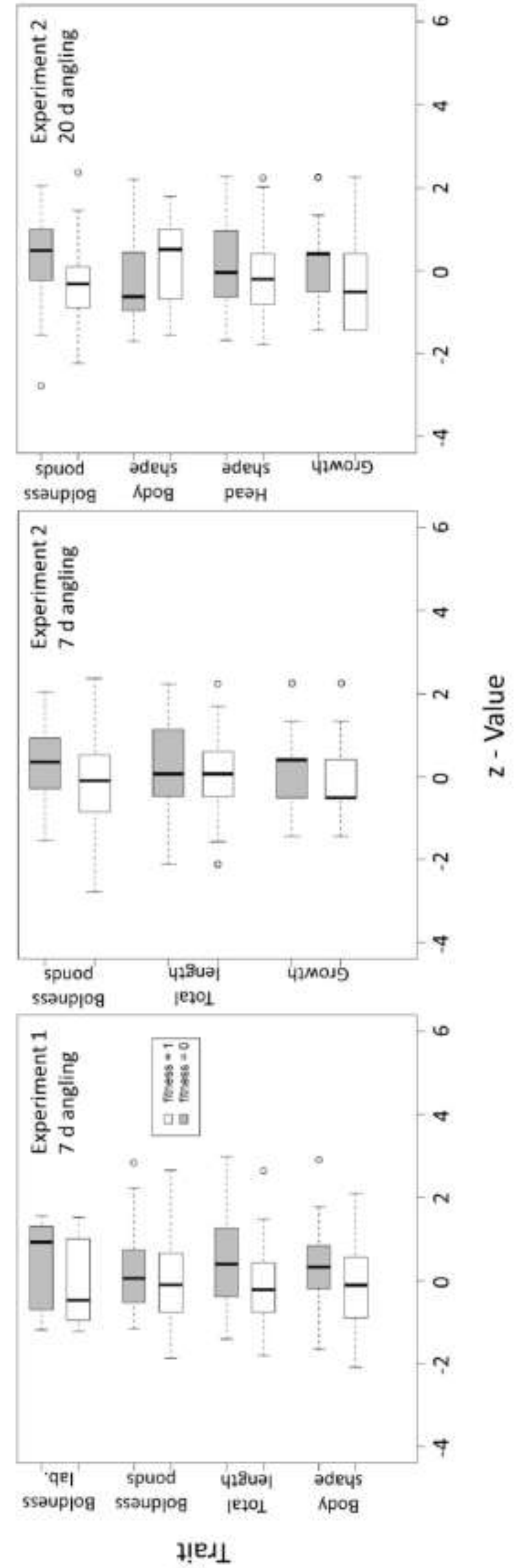
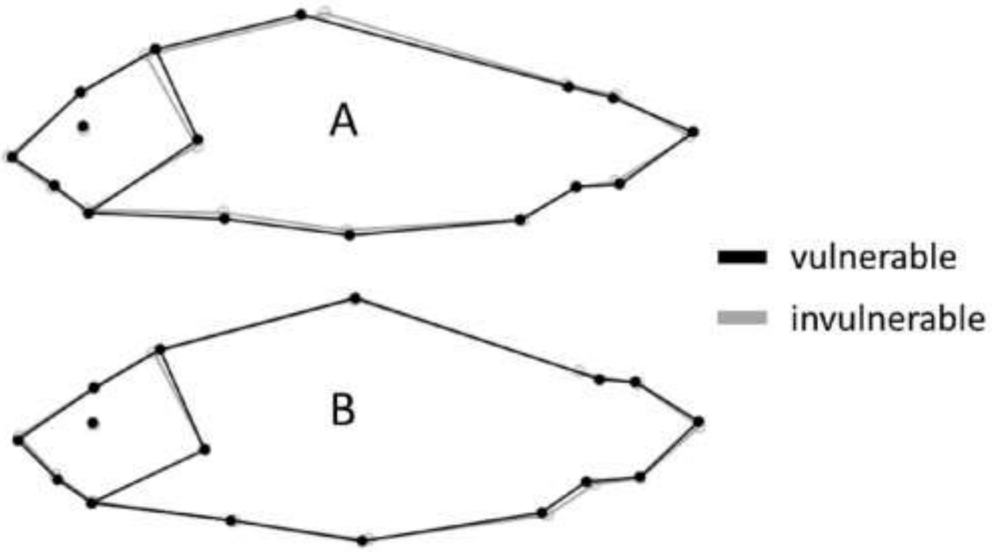

Experiment 1
7 d angling
fitness = 1
fitness = 0
Boldness lab.
Boldness ponds
Total length
Body shape
Experiment 2
7 d angling
Boldness ponds
Total length
Growth
Experiment 2
20 d angling
Boldness ponds
Body shape
Head shape
Growth
-4
-2
0
2
4
6
z - Value
Trait

Fig. 4



Appendix 1

Personality Assessment in the Laboratory

Before our N = 96 juvenile carp were tested for their behavioral expressions under laboratory conditions within two contexts (see main text) a subsample of N = 36 fish was tested for the presence of consistent among-individual differences in behavioral traits (defined as personality) as derived from four separate trials conducted within four different contexts to finally select the most meaningful tests that were then applied on all 96 experimental fish. All experiments were conducted under standardized laboratory conditions in aquaria on individual focal carp. Experiments aimed at capturing three salient temperament traits – exploration (an individual`s reaction to a new situation), boldness (an individual`s reaction to any risky situation, but not new situation) and sociability (an individual`s reaction to the presence or absence of conspecifics) following Réale et al. (2007).

Experimental setup

Six aquaria (130 cm x 40 cm x 40 cm, 208 L) were placed within a temperature-controlled climate chamber (ILKAZELL, inner dimensions: 276 cm x 210 cm x 176 cm, ILKAZELL Isoliertechnik GmbH, Zwickau, Germany) with a constant temperature of 20 °C and used for repeated behavioral assays on individual focal carp. Each tank consisted of a central open area compartment (80 cm x 40 cm x 40 cm, arena), bordered by a refuge compartment (25 cm x 40 cm x 40 cm, refuge) and a shoal compartment (similar to refuge compartment, shoal, Fig. A1-1). The refuge was separated from the arena by an opaque black plastic barrier equipped with a sliding door. The sliding door (20 cm x 20 cm) could be lifted by a remote pulley system from outside the climate chamber (Fig. A1-1). The shoal compartments were stocked with four conspecifics (comparable size to the focal fish) and were used to test for sociability of the focal fish (see shoaling trial below). Once stocked, the conspecifics within the shoal compartment did not change over the course of the experiment. The shoal compartment was separated from the arena by a transparent PLEXIGLAS® (Rhön GmbH, Darmstadt, Germany) barrier equipped with a one way window film. Hence, the focal fish could see the shoal only when extra lighting (60 W spotlights) was applied within the shoal compartment, which was only done in the specific sociability test (see below).

All four behavioral assays were remotely conducted from outside the climate chamber using remote pulley systems and video cameras (OSCAR CCD Camera, 640 x 480 pixel), placed in front of each aquaria. Focal fish were stocked into the closed aquaria refuge on Thursdays or Fridays and allowed to recover over the weekend. Behavioral measurements were then conducted on 4 consecutive days using the same fish from Monday until Thursday. During the experimental week and the weekend before, all focal fish in the refuges of the aquaria were fed once every 24 hours (carp pellets, 2 mm diameter, Aller Classic, Aller Aqua, Golßen, Germany) with an amount of 1 % of the fish`s body mass using automatic feeding machines (EHEIM Futterautomat TWIN, EHEIM GmbH & Co. KG, Deizisau, Germany). Feeding was always conducted at the same time and stopped automatically at 15 hours before the onset of the experiment to control for hunger levels. In all experimental contexts, behavior was quantified only in the arena, not in the refuge, following the methods of Wilson and

Godin (2009). On the first experimental day, all fish were tested for their exploration behavior because this test demands a novel environment (Réale et al. 2007). The sequence of the other contexts was randomized. In every context, fish were given 60 minutes to leave the refuge followed by a ten minutes observation period within the arena. All tests were repeated with each focal fish after exactly six weeks to be able to estimate consistency of individual variation in behaviors.

Experimental processes of behavioral observations within the laboratory

Exploration (Context 1): Individual carp were tested for their willingness to explore a novel environment following Wilson and Godin (2009). Upon trial start, the trap door separating the refuge compartment from the central open area compartment was lifted. Thereby, the fish were allowed to enter and explore the arena (Fig. A1-1 "A"). Afterwards each fish was given 60 minutes to leave the refuge and to investigate the arena. If a fish did not leave the refuge after 60 minutes, it was assigned to the maximum score of "60". The time to leave a refuge is widely used as a measure for exploration (Réale et al. 2007) and/or boldness (Brown et al. 2005, Wilson and Godin 2009). In the present study, following Réale et al. (2007), time to leave a refuge was presumed to be a measure for exploratory behavior because it is measured in a novel and potentially risky environment (whereas boldness is to be assessed in non-novel environments, see Réale et al. 2007). If the fish left the refuge, the time spent within the upper water zone of the aquaria (defined as being within the closer 50 % of the water column towards the surface) was measured as an additional indication of boldness within this exploration trial (Wilson and Godin 2009). If a fish did not enter the upper water zone, it was assigned to the minimum score of "0".

Exploration of a novel object (Context 2): Fish were tested for their willingness to approach a novel object in the arena following Frost et al. (2007). This experiment is assumed to test for boldness because inspection of the novel object is potentially risky (Wilson et al. 1993, Frost et al. 2007). The novel object was placed beforehand in the center of the middle third of the arena, visible from inside the refuge after opening the door (Fig. A1-1 "B"). The novel object was constructed as a yellow cuboid of 10 cm x 6.5 cm x 3.2 cm (LEGO Group, Billand, Denmark), following Frost et al. (2007). Apart from the presence of the novel object in the center of the arena, the setup was identical to context 1, with the difference that the fish already knew the arena. The fish was given 60 minutes to enter the arena and explore the novel object. Upon entry, a 10 minutes observational period began. Proximity to the novel object, defined as the time spent within one fish length of the novel object was recorded as a measure for risk-taking behavior towards a novel object. If a fish did not explore the novel object, it was assigned to the minimum score of "0".

Feeding and feeding under disturbance (Context 3): The fish`s willingness to feed under risk and to resume feeding after a fright stimulus was tested on the basis of Ward et al. (2004) and Álvarez and Bell (2007). The underlying assumption was that individuals differ in risk-taking behavior in terms of feeding under risk (Réale et al. 2007) and in recovery speed after a fright stimulus (Ward et al. 2004), both being assumed to be measures of boldness. The general setup for this experimental context followed context 1. In addition, an apparatus was fitted above the center of the arena, containing a mechanism that allowed the controlled release of a food pellet simultaneously to the opening of the sliding door (Fig. A1-1 "C"). The fish was given 60 minutes to exit the refuge. Once the fish was in the arena, it was given 10 minutes to find and consume the first food pellet. Once the focal fish was observed to feed, we again pulled the line of the apparatus above the arena, and a 40 g led angling weight and another pellet were released together, initializing a fright stimulus for the fish (which was found to be effective in a pilot study) and providing additional food jointly (Fig. A1-1 "C"). To prevent the weight from dropping onto the bottom of the glass-aquaria, it was stopped with a thin line (a 3 lbs monofilament fishing line) after plunging 15 cm deep into the water. The time span between triggering of the fright stimulus and consumption of the second pellet was again measured and was considered a second measure for risk-taking similar to boldness assessment in fishes in other studies (Ward et al. 2004, Álvarez and Bell 2007). If a fish did not consume one of the pellets, time to feed was coded as "0".

Shoaling (Context 4): Following Ward et al. (2004) and Krause and Ruxton (2002), individual carp were tested for their willingness to associate with a stimulus group of conspecifics (Fig. A1-1 "D"). For this purpose the additional overhead spotlight above the shoal compartment was switched on so that the focal fish could see the shoal. Shoaling tendency has been considered a measure for sociability in other studies (Ward et al. 2004) and was quantified by recording the amount of time that the focal fish spent within an association zone, defined as within 2 fish lengths of the one-way glass separation. Over a period of 10 minutes, provided the focal fish emerged from refuge within 60 min, sociability of the fish was enumerated. If a fish did not leave the refuge, time spent within the association zone of the novel object was coded "0".

Statistical analyses

Statistical analysis were designed to identify i) correlations among the behavioral measures assessed in the four trials to derive personality traits as measured by one or more of the behaviors described above and b) the repeatability of personality-related behaviors as evidence for the existence of personality traits (i.e., consistent individual differences in behavioral traits) (Dingemanse and Wolf 2010). In relation to i), suites of correlated behaviors were identified using principle component analyses (PCA) with varimax rotation for improved interpretation of factor structures (Quinn and Keough 2002). The idea was that factors emerging in the PCA would sum correlated behavioral measures that would then be interpretable as distinct personality axes (e.g., exploration, boldness, sociability). PCA were conducted separately for the first and the second trials (six weeks later) of experimentation using all measures that were assessed in all four behavioral trials. Variables used for the initial PCA were "Time to emerge from refuge" (exploration trials), "Time spent in upper water zone" (exploration trial), "Time spent within one fish length of a novel object" (novel object trial), "Time to ingest the 1st food item" (feeding trial), "Time to ingest the 2nd food item" (feeding trial) and "Time spent within two fish lengths of shoal" (shoaling trial). Behavioral measures with a minimum factor loading of 0.5 were considered meaningful for component interpretation (Peres-Neto et al. 2003). All values were standardized to a mean of 0 and a SD of 1 before conducting the PCA. The PCA analysis was repeated using the same parameters from the second observation period six weeks later.

After identifying internally consistent factors in the first PCA the repeatability of behavior comparing the first and the second observation period six weeks later using the factor structure identified in the first trial was estimated following Lessells and Boag (1987). We also calculated the rank order consistency using Spearman rank correlations. For each factor (as determined by PCA) we summed z-scores of behavioral variables loading heavily on specific PCA axis to create the behavioral index. Factor structures as revealed by PCA in the first trial were applied to the data of the second observation period because we did not find the same variables loading on the same axis in the two initial PCAs. Because habituation effects could explain this pattern, we focused on the first PCA to identify correlations among behaviors.

Factor scores of exploration behavior and boldness related behavior for all final N = 94 individuals (see main text) were used to test whether laboratory behavior would be predictive of angling vulnerability in ponds (see main text). These factor scores were used to test for correlation analyses between exploration and boldness behavior from the laboratory and comparable z-standardized measurements of behavior within the ponds (use of shelter structure and visits at the close and the distant feeding spot) using Pearson`s correlations.

Results

The PCA using data from the first trial (N = 36 fish) revealed two principal components, explaining 46.0 % and 22.2 % of total variance, respectively. PCA 1 incorporated the „Time spent within one fish length of a novel object", "Time to ingest the 1st food item" and "Time to ingest the 2nd food item", and hence joint variables supposed to indicate boldness-related behaviors (Tab. A1-1). PCA 2 was composed of the variables "Time to leave the refuge", "Time spent in upper water zone", and "Time spent within 2 fish lengths of shoal" supposed to indicate exploration and sociability. We labelled the PCA 1 "Boldness" and PCA 2 "Exploration/Sociability". The PCA from the second trial six weeks later differed substantially from these results, already indicating low behavioral consistency of the fish between the two trials. Again two principal components were found, together explaining 59.8% of total variance (35.3 % and 24.5 % respectively). PCA 1 was composed of the variables "Time to leave the refuge", "Time to ingest the 1st food item", and "Time to ingest the 2nd food item". PCA 2 was composed of the variables "Time spent within 2 fish lengths of a novel object", "Time spent within 2 fish lengths of shoal", and "Time spent in upper water zone" (Table A1-1). Hence, both factors were not clearly interpretable in the second trials.

Spearman rank correlations between summed z-scores of the two trials were insignificant for both factors (Table 2 of the main manuscript, Fig. A1-2) indicating low behavioral consistency of our experimental fish over time. However, the p-values for PCA 1 (boldness) was close to significance (P = 0.064, Table 2 of the main manuscript). Calculation of repeatability from summed z-scores of variables loading on the respective PCA resulted in a moderate, yet insignificant, repeatability estimate of r = 0.26 for PCA 1 and an insignificant of r = 0.15 for PCA 2. Underlying F-statistics were not significant in both cases (Table 2 of the main manuscript), although again there was a trend for the boldness measure to be close to significance (P = 0.060). In fact, PCA 1, consisting of the „Time spent within one fish length of a novel object", "Time to ingest the 1st food item", and "Time to ingest the 2nd food item", which are indicative of boldness related behaviors, showed a remarkably higher repeatability estimate than PCA 2 (Table 2 of the main manuscript). Therefore, we found only weak evidence for a stable personality trait in laboratory-assayed carp, but could tentatively conclude that potentially there was a trend for boldness being repeatable. We thus included the boldness-related laboratory behavior in our predictive models to explain angling vulnerability (see main text) and also examined the relationship of laboratory boldness behavior and pond boldness behavior using all final N = *94* fish (Fig. A1-3 and compare main text).

Appendix 1 References

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Tables Appendix 1

Tab. A1-1: Component loadings of behavioral measures from trial A (A) and trial B (B) on two orthogonally rotated principal components with N = 36 carp.

A

Behavioral trait trial (A)	Principal Components	
	PCA 1	PCA 2
Time to leave the refuge (Exploration)		-0.891
Time spent in upper water zone (Exploration)		0.897
Time spent within 2 fish lengths of shoal (Shoaling)		0.501
Time spent with novel object (Novel object)	-0.713	
Time to ingest 1st food item (Boldness)	0.782	
Time to ingest 2nd food item after disturbance (Boldness)	0.838	
Eigenvalue	2.76	1.33
Variance explained %	46.0	22.2

B

Behavioral trait trial (B)	Principal Components	
	PCA 1	PCA 2
Time to leave the refuge (Exploration)	0.830	
Time spent in upper water zone (Exploration)		-0.556
Time spent within 2 fish lengths of shoal (Shoaling)		0.736
Time spent with novel object (Novel object)		0.736
Time to ingest 1st food item (Boldness)	0.637	
Time to ingest 2nd food item after disturbance (Boldness)	0.875	
Eigenvalue	2.12	1.47
Variance explained %	35.3	24.5

Figures Appendix 1:

Fig. A1-1: Experimental setup of our four behavioral tests under laboratory conditions using N = 36 juvenile carp. A = Exploration, B = Novel object, C = Feeding and feeding under disturbance, D = Shoaling.

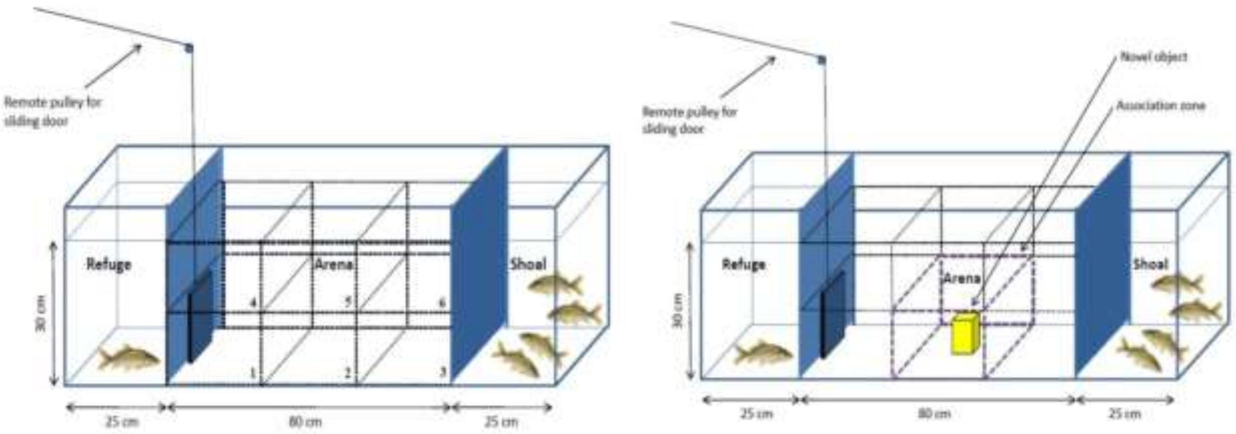
Fig. A1-2: Scatter plots of Summed z-scores from behavioral observations revealed to load on the same factor in PCA 1 from the first trial and applied to data from the second trial six weeks later. A = PCA 1, B = PCA 2.

Fig. A1-3: Scatter plots of PCA1 (boldness) and PCA2 (exploration) as determined from behavioral observations under laboratory conditions using all N = *94* carp (see main text) and z-standardized boldness-related behavioral observations from the pond environment (shelter usage, visits at the close and the distant feeding spot).

Fig. A1-1

A – Exploration

B – Novel object



C – Feeding and feeding under risk

D - Shoaling

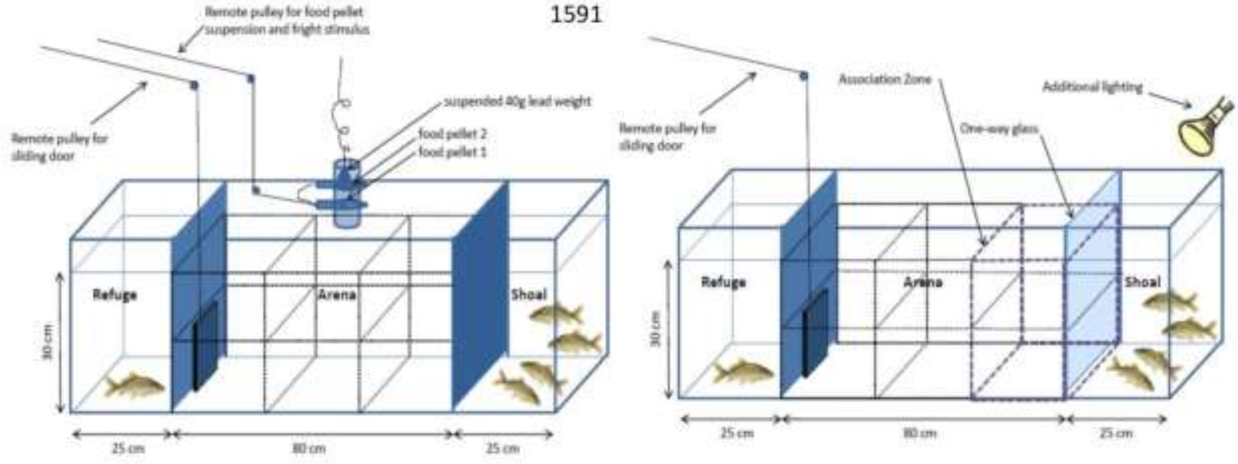
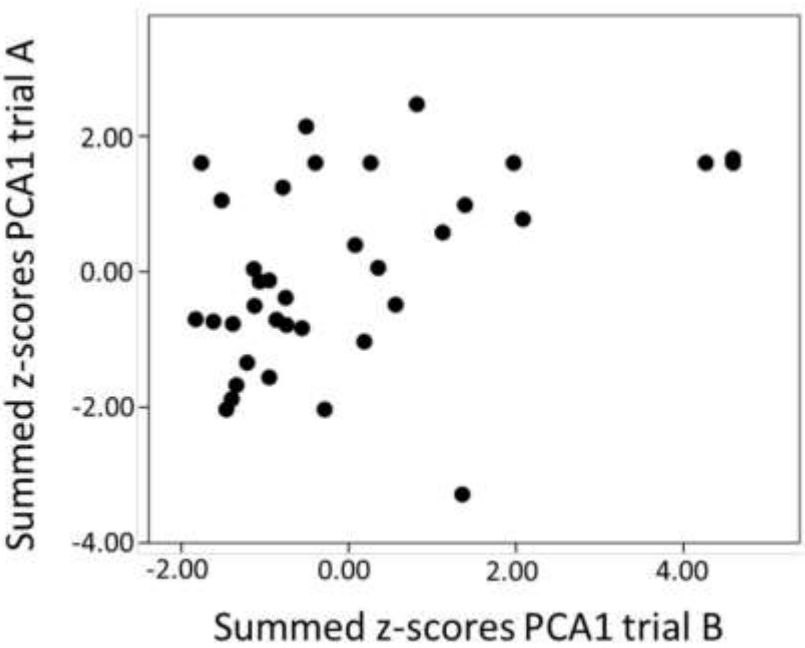


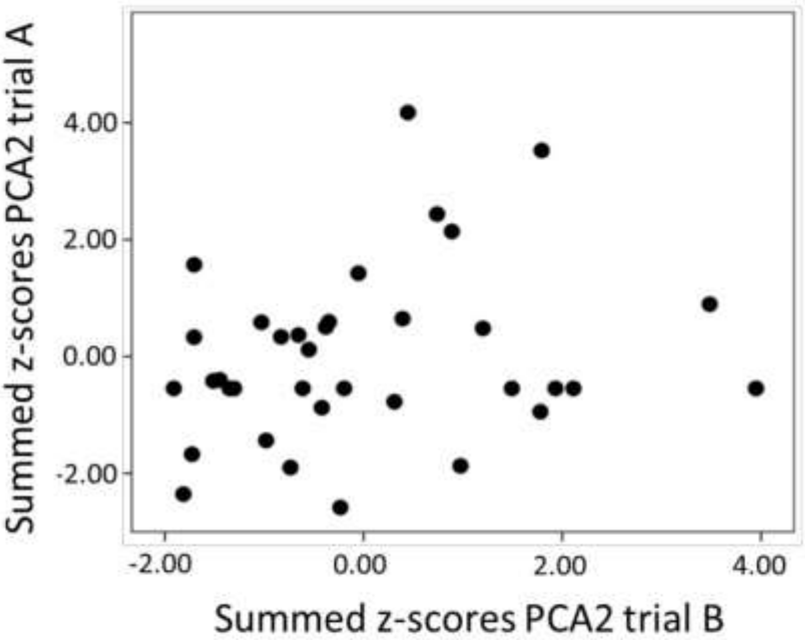
Fig. A1-2

A



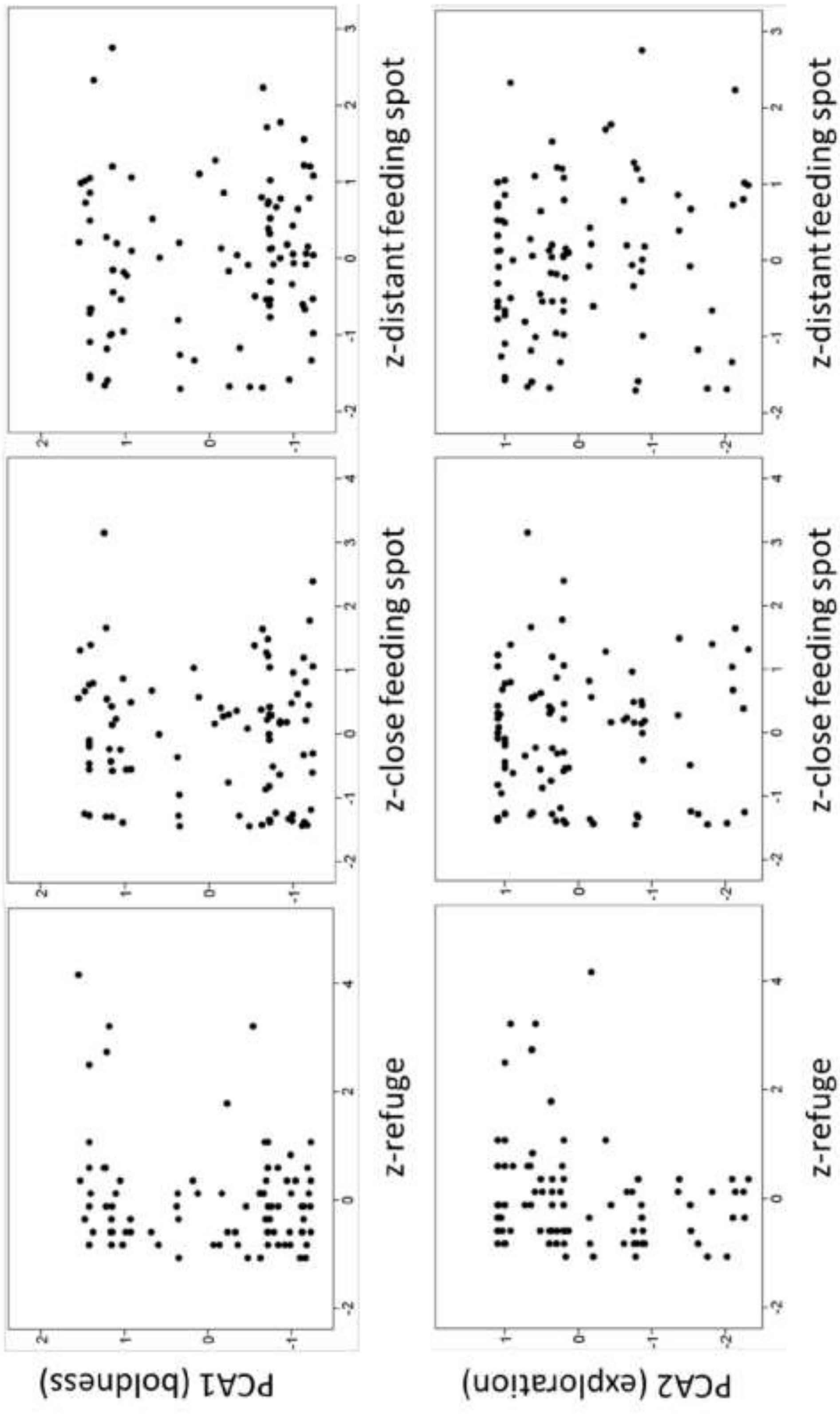
2.00
0.00
-2.00
-4.00
-2.00
0.00
2.00
4.00
Summed z-scores PCA1 trial A
Summed z-scores PCA1 trial B

B



4.00
2.00
0.00
-2.00
-2.00
0.00
2.00
4.00
Summed z-scores PCA2 trial A
Summed z-scores PCA2 trial B

Fig. A1-3


PCA1 (boldness)
PCA2 (exploration)
z-refuge
z-close feeding spot
z-distant feeding spot
-2
-1
0
1
2
3
4

Appendix 2

Calibration of the PIT system

Calibration of the PIT system used in this study was conducted within a large laboratory tank. The size of the tank (10 m x 4 m x 1 m, L x W x H) and PIT system installation within the tank was similar to the setup used by Klefoth et al. (2012) and comparable to the setup within ponds used in the present study (compare Figure 1 of the main manuscript). The PIT system covered two feeding spots (0.5 m diameter) and a shelter structure (2 m x 4 m). PIT antennae covering the feeding spots were round circle antennae where the antenna cable was bound to a circle and placed directly on the bottom of the tank. Two antenna loops (1 m x 4 m) were installed in front of the shelter structure and covered the complete width and height of the tank. We used N = 40 carp (TL ± SD = 228 ± 19.6 mm) tagged with PIT tags (23 mm, Oregon RFID, Oregon, USA) to calibrate our passive telemetry system. Out of the 40 fish, 3 randomly selected individuals were additionally tagged with external floy-tags (Floy Tag Inc., Seattle, Washington, USA) for external visual identification. The first individual was floy-tagged at the edge of the left pectoral fin (ID 348), the second individual was marked at the edge of both pectoral fins (ID 435), and the floy tag for the third individual was placed at the ventral point of maximum curvature of the peduncle (ID 351). These additional external tags allowed to identify three individuals to be present at the two feeding spots or to be present within the shelter structure, irrespective of the automatic PIT system and were therefore used for calibration of the PIT system.

Three cameras (Oscar CCD Camera 640 x 480 Pixel) were installed to visually observe the fish. Camera 1 and camera 2 were placed above the close and the distant feeding spot (approximately 0.5 m above the water surface) and camera 3 was installed at a window glass at the side of the tank where antennae loops in front of the shelter structure were located. On four consecutive days all N = 40 fish were simultaneously observed using the PIT system and the three cameras for one hour each. We followed the behavior of our three externally tagged individuals using the video data and compared the data with those collected from the automatic PIT system. Whenever an externally tagged carp entered one of the feeding spots (i.e. the fish was found to be within the antenna loop with a minimum of 50 % of its body length), we counted the maximum number of additional conspecifics within the feeding spot and measured the time spent within the antenna loop. Whenever one of the three focal fish reached one of the two antenna loops in front of the shelter structure we again counted the number of conspecifics present, but did not measure the time spent there as the single antenna loops in front of the shelter were only crossed during our observation periods and the focal fish did not stay on top of the antennae. We defined a fish to be sheltering after it had passed the antennae loops in a direction from outside to the inside of the shelter. Sheltering activities ended when the fish passed the antennae loops in the opposite direction or when it was detected elsewhere. Based on our video data we took eight randomly selected time frames of 10 minutes and stopped the time spent sheltering of our focal fish within this time frame. If a focal fish did not leave the shelter after the ten minutes, observation time was elongated until sheltering activities ended. We then compared theses real observations (min. spent sheltering) with our PIT data (min. spent sheltering).Similar comparisons were done for the feeding spots based on 18 randomly selected time frames of 10 minutes. Here, the number of visits at the feeding spots were counted on video data and compared with PIT data.

Statistics

To examine the functionality of the PIT system, logistic regression analyses were used. An individual observation was coded one if both, visual observations and the PIT system revealed a fish to be present within one of the structures (feeding spot, shelter structure). An observation was coded 0 if only the visual observation, but not the PIT system indicated a structure use of the fish. Visits at the close and the distant feeding spot were grouped as preliminary analyses revealed no differences in detection efficiency between these two similar structures. The number of conspecifics present at the same structure and the time (s) spent within the proximity of the antennae (only feeding spots) was added to the model as explanatory variable. The real time (min) spent sheltering and the real number of visits at the feeding spots as indicated by visual observations was compared with the time spent sheltering and the number of visits at feeding spots as calculated based on PIT data using Pearson`s correlation. Single events of specific habitat choice of the three externally marked fish were treated as independent observations. Analyses were conducted using software package R version 3.1.2 (R Development Core Team) by applying library MASS (Venables and Ripley 2002).

Results

During our one hour observation periods over four days we found the three focal fish to visit the feeding spots a total of 77 times. The mean time spent on the feeding spots was 3.1 ± 2.6 s (range 1 - 16 s) and the mean number of fish present was 3.2 ± 2.0 (range 1 – 10). The antennae in front of the shelter structure were crossed 395 times and the mean number of 5.0 ± 3.0 fish were present (range 0 – 15). The logistic regression analyses revealed the number of conspecifics present within the vicinity of the antennae to be the main predictor of PIT detection (Tab. A2-1, Tab. A2-2, Fig. A2-1, Fig. A2-2) whereas the time spent within the circle antennae loops did not influence detection probabilities (Tab. A2-2). Visual observations in comparison with our PIT data revealed a detection probability of 68.8 % over all cases at the circle antennae with a 100 % probability of detection if the focal fish was alone and continuously lower detection probabilities with an increasing number of conspecifics in the vicinity of the antennae (Tab. A2-1, Fig. A2-1). Our additional ten minutes observation periods of feeding spots (N = 18) revealed a high correlation between PIT system data and visual observations (Pearson`s correlation, T = 5.741, df = 16, r = 0.820, P < 0.001, Fig. A2-3). Similar observations of sheltering activities (N = 8) underestimated the time spent sheltering by factor 4.4 [mean time spent sheltering (min) ± SD based on visual observations and calculated from PIT data, 6.70 ± 2.84 and 1.52 ± 1.91 min, respectively], but were highly correlated with the real time spent sheltering as determined visually (Pearson`s correlation, T = 3.169, df = 6, r = 0.791, P = 0.019, Fig. A2-4). Therefore, the automatic PIT system revealed reliable data to observe the number of visits at feeding spots and the time spent sheltering within our experimental setup. However, the system was influenced by the number of conspecifics present and circle antennae on the bottom as used for covering the feeding spots revealed better results compared to antenna loops in front of the shelter structure.

References Appendix 2

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Tables Appendix 2

Tab. A2-1: Descriptive data of our calibration experiments. Presented are the total number of fish present when one of the focal fish used a PIT covered structure and the number of successful detections in dependence of conspecifics occurrence.

Number of fish present	Number of observations	Positive detections	No detections	Proportion of positive detections %
@ Feeding spots				
1	16	16	0	100
2	16	13	3	81
3	20	13	7	65
4	9	7	2	78
5	6	2	4	33
6	3	2	1	67
7 - 10	7	0	7	0
@ Shelter structure				
1	33	28	5	85
2	50	23	27	46
3	60	7	53	12
4	50	1	49	2
5	59	4	55	7
6	43	1	42	2
7 - 16	100	0	100	0

Tab. A2-2: Logistic regression modelling of carp detection along two different types of structures (feeding spots and shelter) using an automatic PIT system within a laboratory tank. Independent parameters were the number of conspecifics present within the vicinity of the PIT antenna and the time (s) spent on feeding spots.

Parameter	Estimate	SE	z-value	P
Feeding spots				
Conspecifics	-0.76	0.19	-3.93	< 0.001
Time	0.18	0.16	1.12	0.261
Shelter				
Conspecifics	-1.29	0.17	-7.68	< 0.001

Figures Appendix 2

Fig. A2-1: Probability of positive detection on feeding spots using an automatic PIT system and circle antennae placed at the bottom of a large tank in dependence of the number of conspecifics present.

Fig. A2-2: Probability of positive detection in front of a shelter structure using an automatic PIT system and antenna loops in dependence of the number of conspecifics present.

Fig. A2-3: Correlation between the real number of visits at the feeding spots during N = 18 ten minutes lasting periods as observed visually and the number of visits at the feeding spots as determined by PIT data.

Fig. A2-4: Correlation between the real time spent sheltering (min) during N = 8 ten minutes lasting periods as observed visually and the time spent under shelter (min) as determined by PIT data.

Fig. A2-1:

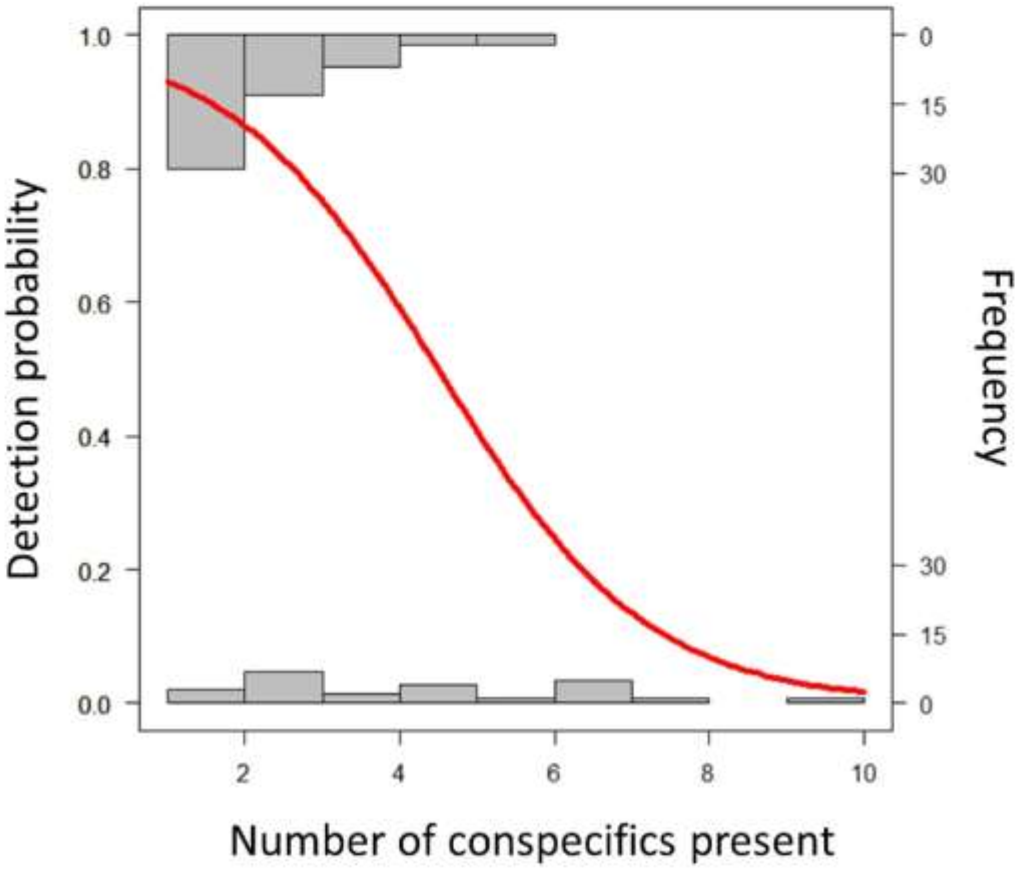

Detection probability
1.0
0.8
0.6
0.4
0.2
0.0
Frequency
0
15
30
30
15
0
2
4
6
8
10
Number of conspecifics present

Fig. A2-2:

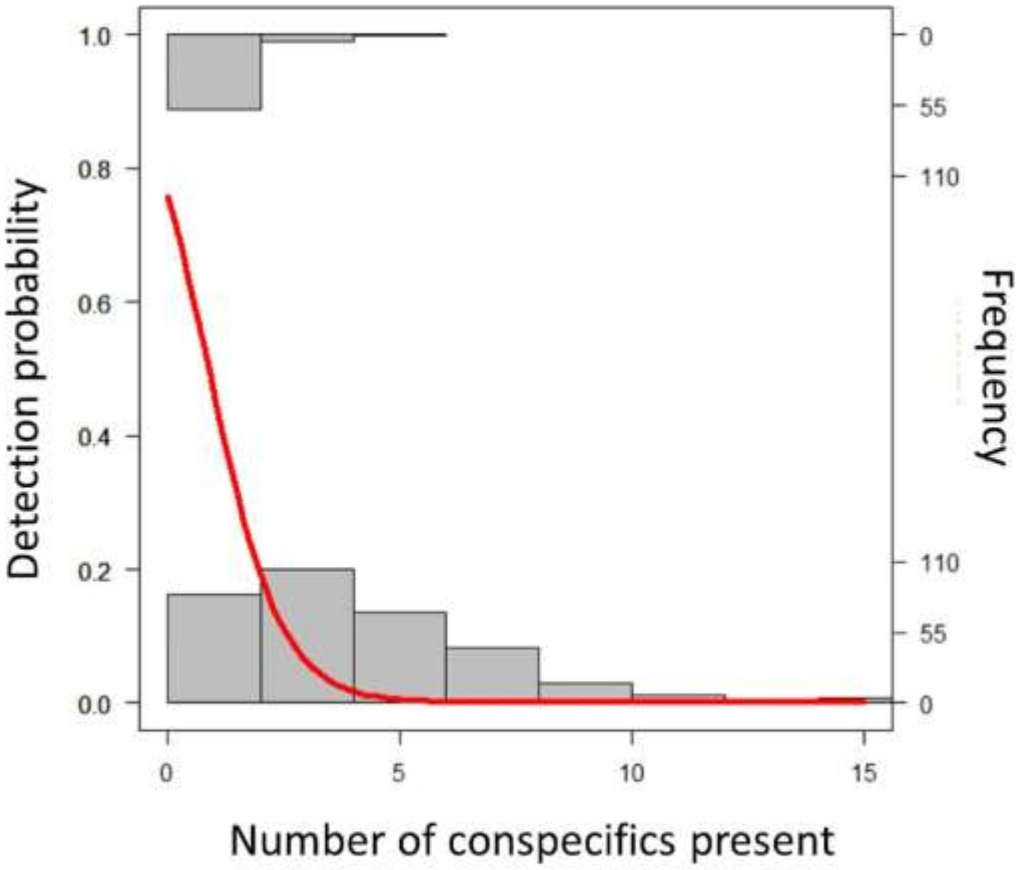

Detection probability
1.0
0.8
0.6
0.4
0.2
0.0
Frequency
0
55
110
110
55
0
0
5
10
15
Number of conspecifics present

Fig. A2-3:

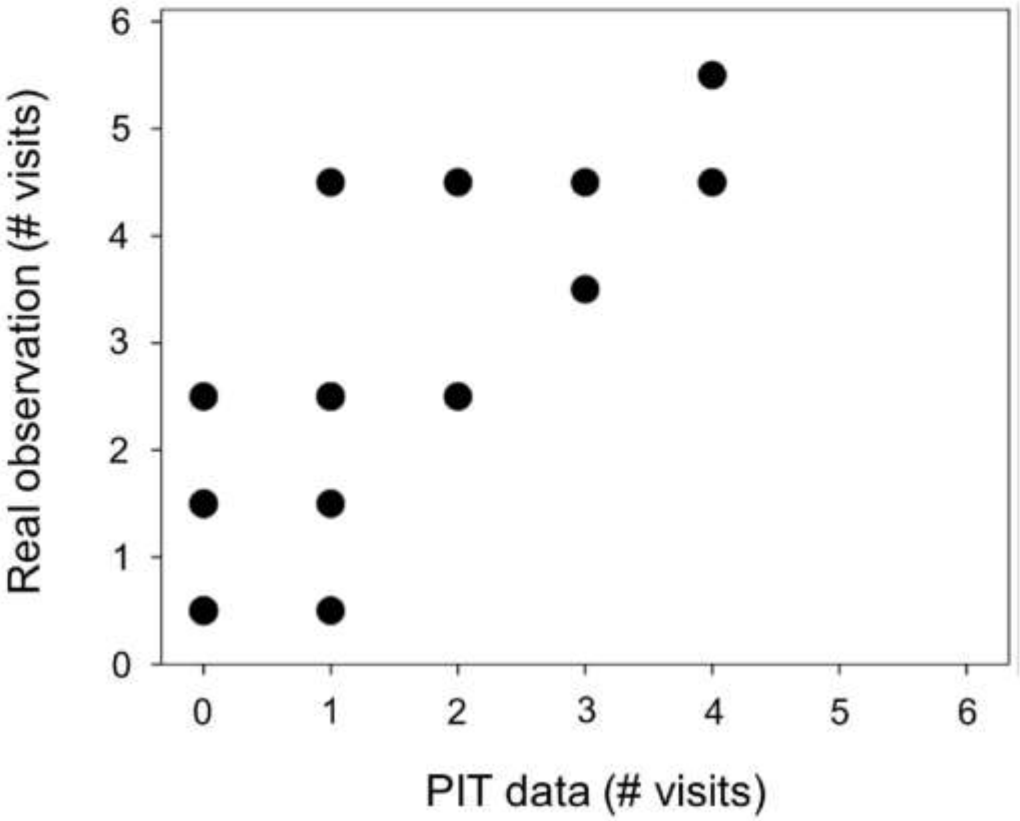
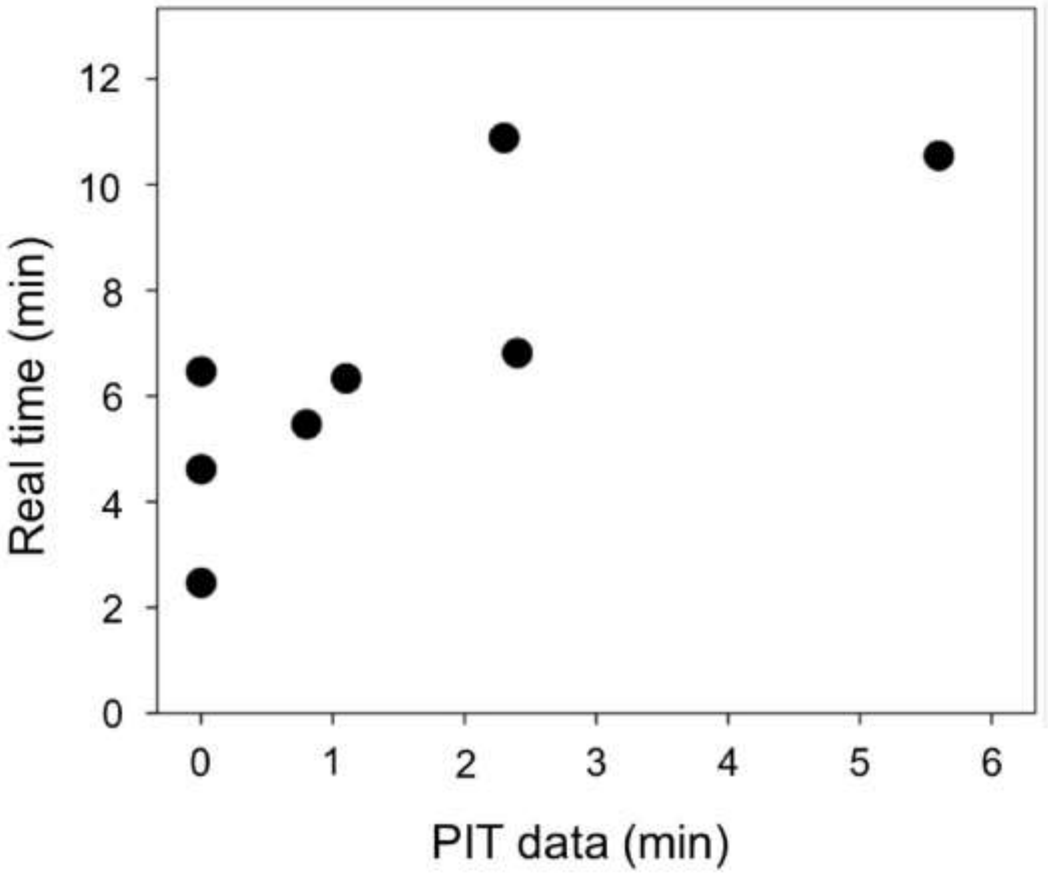


Fig. A2-4:



Paper V

V

Jun-ichi Tsuboi, Kentaro Morita, Thomas Klefoth, Shinsuke Endou, Robert Arlinghaus, 2016. Behaviour-mediated alteration of positively size-dependent vulnerability to angling in response to historical fishing pressure in a freshwater salmonid. Canadian Journal of Fisheries and Aquatic Sciences, 73:461-468.

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Behaviour-mediated alteration of positively size-dependent vulnerability to angling in response to historical fishing pressure in a freshwater salmonid

Jun-ichi Tsuboi, Kentaro Morita, Thomas Klefoth, Shinsuke Endou, and Robert Arlinghaus

Abstract: Positively size-selective vulnerability to fishing is well established in recreational fisheries. Size-selective harvesting can either induce an indirect selection response of behavioural traits that are correlated with size or exert direct selection pressures on behaviours that contribute to vulnerability. In addition, learning to avoid future capture may always happen. Behavioural change caused by fishing may in turn affect the size-selective properties of angling. To test this prediction, field experiments with amago salmon (*Oncorhynchus masou ishikawae*) were conducted. We demonstrated that, as expected, large fish were more vulnerable than smaller individuals in a low fishing pressure (LP) stream and that positively size-selective angling was not (or no longer) present in a high fishing pressure (HP) stream. Moreover, fish in the HP stream were by far less vulnerable to angling with natural bait than fishes in the LP stream. Laboratory studies showed that offspring from HP streams were intrinsically shyer than offspring collected from LP streams. We propose that the increased timidity of individuals inhibiting HP streams disrupted the generally positive relationship among salmon body size and its vulnerability to angling. Fisheries-induced timidity in response to high exploitation rates reduces catchability, affects the size-selective properties of angling, reduces the value of fishery-dependent stock assessments, and potentially affects trophic interactions.

Résumé : La vulnérabilité à la pêche positivement reliée à la taille est un phénomène bien documenté dans les pêches sportives. La pêche avec sélection selon la taille peut soit induire une réaction de sélection indirecte de caractères comportementaux qui sont corrélés à la taille ou exercer des pressions de sélection directes sur des comportements qui accroissent la vulnérabilité. En outre, l'apprentissage de l'évitement de captures futures peut toujours se produire. Les changements comportementaux causés par la pêche peuvent, quant à eux, avoir une incidence sur les propriétés de sélection selon la taille de la pêche. Pour vérifier cette prédiction, des expériences sur le terrain avec des saumons amago (*Oncorhynchus masou ishikawae*) ont été menées. Nous avons démontré que, comme prévu, les grands poissons étaient plus vulnérables que les poissons plus petits dans un cours d'eau caractérisé par une faible pression de pêche (LP) et que la pêche avec sélection positive selon la taille n'était pas (ou plus) présente dans un cours d'eau caractérisé par une forte pression (HP). De plus, les poissons dans le cours d'eau HP étaient beaucoup moins vulnérables à la pêche sportive avec des appâts naturels que les poissons dans le cours d'eau LP. Des études en laboratoire ont démontré que la progéniture provenant des HP était intrinsèquement plus timide que la progéniture prélevée des cours d'eau LP. Nous proposons que la timidité accrue des individus dans les cours d'eau HP perturbe la relation généralement positive entre la taille du corps des saumons et leur vulnérabilité à la pêche sportive. La timidité induite par la pêche en réaction à des taux d'exploitation élevés réduit la capturabilité, influence les propriétés de sélection selon la taille de la pêche, réduit la valeur des évaluations des stocks dépendant de la pêche et pourrait avoir une incidence sur les interactions trophiques. [Traduit par la Rédaction]

Introduction

Recreational fishing can induce evolutionary changes in life history, morphology, physiology, and behaviour in response to excessive and (or) trait-selective exploitation (e.g., Uusi-Heikkilä et al. 2008, 2015; Sutter et al. 2012). Most life-history changes caused by intensive fishing collectively reduce adult body size at age (Jørgensen et al. 2007; Alós et al. 2014). Evolutionary downsizing will in turn affect catch rates because most fishing gears operate positively size-dependently and hence more readily catch the larger size classes of an exploited population. Passively operated fishing gears, such as recreational angling or gill-netting, may not only select on body size and life history (Saura et al. 2010; Arlinghaus et al. 2009; Alós et al. 2014; Evangelista et al. 2015), but also select for activity, exploration, aggression, and other behavioural traits (Heino and Godø 2002; Olsen et al. 2012; Diaz Pauli et al.

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2015; Wilson et al. 2015). Changes in body size and correlated behavioural traits over time in response to intensive fisheries can affect individual reproductive success due to the linear relation of body mass and fecundity characteristic for most temperate fishes (Wootton 1992) and the reduced nest vigilance shown by low vulnerable individuals in species that provide parental care (Sutter et al. 2012). One can also expect intensively angling-exploited fish stocks to not only host smaller growing fishes after maturation (Alós et al. 2014), but also individuals revealing increased levels of timidity due to fisheries-induced behavioural evolution and associated hook-avoidance learning effects in catch-and-release fisheries (Raat 1985; Askey et al. 2006; Alós et al. 2012, 2015; Klefoth et al. 2012, 2013).

In most stream salmonids, dominance hierarchies are common, which are usually size-dependent. Large aggressive fish often become dominant, in turn defending territories, which results in greater foraging rates and growth compared with subdominant individuals (Nakano 1995). Therefore, large dominant salmonids are usually more vulnerable to angling under natural conditions compared with smaller subdominant individuals (Tsuboi and Endou 2008). Moreover, for metabolic reasons absolute food consumption positively correlates with size (Yamamoto et al. 1998), in turn leading to larger fish consuming more than small fish, which also elevates capture probabilities of large compared with small fishes (Brauhn and Kincaid 1982). Finally, the mouth gape of large fish facilitates the take of a larger range of lures and bait types (Arlinghaus et al. 2008b), which may further contribute to the greater vulnerability of large fish compared with small fish (Lewin et al. 2006), all other states (e.g., hunger and habitat choice) being equal.

To reach large sizes in highly exploited streams, dominant and bold salmonid individuals need to be able to effectively trade off foraging opportunities and the risk of capture. Boldness, defined as foraging under the risk of predation (Réale et al. 2007), has a genetic basis in salmonids and other fishes (Iguchi et al. 2001; Ariyomo et al. 2013; Dochtermann et al. 2015). Hence, in response to high fishing pressure one would expect freshwater salmonids to become shyer owing to genetic (i.e., fisheries-induced evolution of behavior; Uusi-Heikkilä et al. 2008, 2015) and plastic reasons (i.e., increased hook-avoidance learning; van Poorten and Post 2005; Askey et al. 2006; Klefoth et al. 2012, 2013). When present, increased timidity may decouple the generally present positive relationship of size and vulnerability to fishing across generations (Alós et al. 2015; Januchowski-Hartley et al. 2015). No studies addressing this question exist for wild-living salmonids, but it has been reported that bold and explorative salmonid individuals are preferentially captured by passively operated gill nets and angling gear (Biro and Post 2008; Härkönen et al. 2014).

We tested the vulnerability to angling gear of wild amago salmon (*Oncorhynchus masou ishikawae*) in dependence of their body size and behaviour by comparing individuals inhabiting high angling pressure (HP) and low angling pressure (LP) streams in both natural and laboratory common garden conditions. In the field experiments, we hypothesized that large individuals are more vulnerable than smaller individuals and that fish are particularly hard to catch in a HP stream compared with an LP stream. In associated laboratory experiments, we hypothesized that large fish grow faster and are more vulnerable to angling compared with small fish, but that this effect should be more pronounced in fishes originating from an LP stream. This expectation arose from the idea that the largest fishes of a population that are evolving in a HP stream should be more shy and consequently be less vulnerable to angling compared with similar-sized fish inhabiting an LP stream. Both evolutionary adaptation and learning within the realm of behavioural plasticity may contribute to the very same effect (Alós et al. 2015), but our study was not designed to disentangle among genetic and plastic effects. Instead we aimed at more generally testing the effects of historic fishing pressure on phenotypic expressions and angling vulnerability of a freshwater salmonid using a combined field and laboratory assessment approach.

Table 1. Environmental components of a high fishing pressure (HP) and low fishing pressure (LP) stream.

Stream	Altitude (m)	Stream width (m) (mean ± SD)	No. of litter items left by anglers per hectare (mean, min.–max.)
HP	700	3.73±2.04	33.5, 0–76.9
LP	1160	2.92±1.00	2.7, 0–10.9

Material and methods

Field experiment

To compare the body size-dependent vulnerability to angling between fishes in HP and LP streams, experimental angling was conducted in the Toiwa and Itajiki streams, both belonging to the Fuji River system in central Japan (35°45′N, 138°35′E; Table 1). In both streams, amago salmon is the only resident fish species exhibiting a nonanadromous life history. The Toiwa stream is heavily exploited by anglers because of easy access on paved roads running alongside the stream bank (hereinafter referred to as HP stream). By contrast, the Itajiki stream is located more than 5 km away from the nearest car stop and historically received low fishing pressure (hereinafter referred to as LP stream). Anecdotally, old local anglers interviewed by the first author reported that even in 1950s, it took an hour to reach the HP stream and almost 6 h to reach the LP stream from downtown of the nearest city by bicycle and trekking, underlining the difficult access to the LP stream during the last decades. Corresponding with the historical fishing pressure, during our experimental angling in the years 2008 and 2009, we regularly observed anglers in the HP stream, whereas we met only one angler over a period of 20 days in the LP stream. Moreover, the assumption of historically divergent fishing pressure was supported by the degree of angling-related litter we observed at the stream banks per hectare in the same sections as the ones where we also conducted the angling experiment (Mann–Whitney U test, $p = 0.023$; Table 1). The distance between the two study rivers is about 8.5 km, and both rivers belong to the same catchment and have thus been colonized by same native population of amago salmon until isolation by erosion-control dams in the 1960s (Endou et al. 2006). Angling regulations are identical on both streams (no bag limit and a minimum length limit of 150 mm), and no stocking has been reported for both streams (Tsuboi et al. 2013).

In August 2008 and June 2009, angling experiments were conducted by two to three experimental anglers in three randomly selected sections in each stream, using a 5 m pole (long rod without a reel), a 1 lb line (1 pound = 0.453 kg), and a 0.5 g sinker equipped with a small barbed hook (gape widths of 5.0 mm) to which a stonefly larva was attached as natural bait (Table 2). Fork length and otolith age (from 0 to 4 years old) were measured for each fish caught by angling and also by electrofishing in the first pass (see below). A generalized linear model (GLM) with a binomial distribution of errors was used to compare the relationships between fork length and the vulnerability to angling between the two streams. A generalized additive model (GAM; Zuur et al. 2009) was also used to assess possible nonlinear effects of fork length on angling vulnerability.

Sampling of individuals invulnerable to angling tactics within the river sections was conducted using electrofishing with two passes on the next day of each experimental angling session. These data were also used to assess the population densities. The number of individuals invulnerable to angling in each stream were assessed using the removal method (model M(b), program CAPTURE; White et al. 1978; available from www.mbr-pwrc.usgs.gov/software/index.html; Table 2). Population densities in each stream

Table 2. Summary of amago salmon angling experiments in a high fishing pressure (HP) and low fishing pressure (LP) stream.

					No. of caught fish by electrofishing		
Stream	Date of bait angling	Stream length of angling experiment (m)	No. of fish caught by bait angling*	CPUE (individuals·angler-hour^{-1}) (mean, min.–max.)	First pass*	Second pass	Density of salmon (individuals·m^{-2}) (mean, min.–max.)
HP	8 Aug. 2008	1013	15	0.91, 0.55–1.27	214	121	0.129, 0.087–0.183
	27 June 2009	456	7	0.96, 0.50–1.43	208	70	0.165, 0.134–0.195
LP	7 and 10 Aug. 2008	594	35	2.14, 0.75–4.17	90	45	0.188, 0.110–0.275
	26 June 2009	539	16	2.12, 1.50–3.00	174	52	0.160, 0.158–0.163

*Sampled for anatomy.

just before angling were defined as the sum of the number of individuals angled and the estimated abundance invulnerable to angling divided by the area of study sites. Population densities of the surveyed sections were similar between the two streams (Table 2).

Laboratory growth and behavioural assays

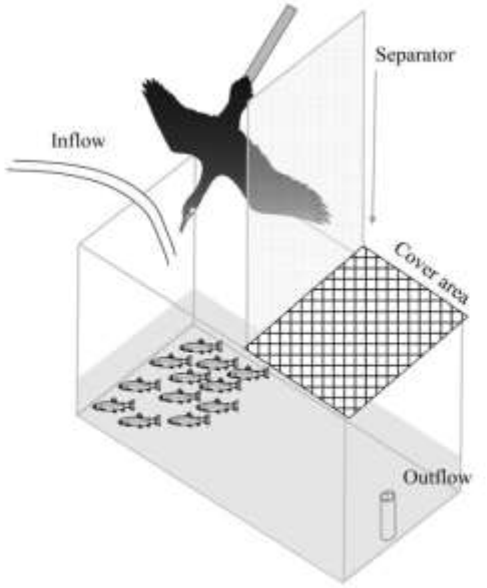
To compare the behavioural characteristics of the LP and HP fish under a common garden, laboratory experiments were conducted using age-0 fish captured at a nonvulnerable fry stage from HP and LP streams. To that end, before the opening of the angling season (1 April 2011), 100 fry of amago salmon were collected using a dip net from the same sections that earlier served as sections for the angling experiment (Table 2; each section about 500 m in stream length) in both the HP and the LP streams on 30 March 2011. We caught fry in all potential habitats and throughout the entire sections. The mean fork length of salmon fry was identical among the streams (HP fish: 30.3 ± 3.2 mm; LP fish: 30.4 ± 2.8 mm; Mann–Whitney U test, $p = 0.952$). The fry were probably not exposed to any fishing activity from birth because their habitat (shoreline leaves in low flow) is entirely separated from the habitat anglers access during fishing activities (Nakano 1995). Moreover, sampling took place before the fishing season started.

All fry collected in the wild were reared in two artificial tanks (width × length × depth: 55 cm × 90 cm × 45 cm) separated by stream origin. During culture and subsequent experiments (see below), each tank was fed with spring water (0.2 L·s^{-1}). Each day during the holding period, fish were given a formulated amago salmon diet composed mainly of fish meal, vitamins C, E, and eicosapentaenoic and docosahexaenoic acid ("Rescue A" produced by Scientific Feed Laboratory Co., Ltd., Tokyo) to satiation using automatic feeders.

To assess the growth potential of the two groups, on 11 July 2011 (103 days after capture in the wild), 86 HP fish and 95 LP fish were measured for fork length (mm) and body mass (g) and individually marked using visible implant elastomer tags. During capture and individual marking, there was no significant differences in mortality between the stream origin (HP: 14%; LP: 5%; G test, $G = 0.234$; $p = 0.628$). Afterwards, the fish were randomly selected and divided into two replicated groups per strain, and 43 individuals of each replicate were transferred to new tanks ($W \times L \times D$: 32.6 cm × 45.7 cm × 30 cm). During the experimental procedure, fish were fed using the same formulated diet as described above. On 16 August 2011, fork length and body mass were remeasured, and specific growth rates (SGR) were calculated as $100 \times (\ln W_{t2} - \ln W_{t1})/(t2 - t1)$, where W_{t1} and W_{t2} are body mass at times t1 and t2, respectively. A generalized linear mixed model (GLMM) with a normal distribution of errors was used to compare the relationships between fork length and the SGR between the two strains, using tank replicate as random factor.

Starting the day after growth measurements, juvenile fish from HP and LP streams were subjected to two multiple repeated test series aiming at determining differences in expressions of risk-taking (boldness) and angling vulnerability in the laboratory.

Fig. 1. Schematic representation of the predator display test, using a stuffed cormorant as a model of a risky piscivorous bird.



Predator display test

On 17–19 August 2011, predator display tests were conducted in an experimental tank ($W \times L \times D$: 55 cm × 90 cm × 45 cm) that was partially covered with a black plate ($W \times L$: 55 cm × 30 cm). Five to six HP or LP individuals were jointly netted from the holding tank and transferred to the uncovered area in the experimental tank (Fig. 1). To study how the groups of fish reacted to predation risk, 30 s after the transfer a stuffed cormorant as a model of a natural predator was shown to the fish just above the water surface in the uncovered area. Three seconds after the cormorant's display, a separator among the covered and uncovered area was introduced into the tank to separate the individuals that were hidden under the cover from those that stayed in the uncovered area. After each experiment, fish were transferred back to the holding tank and given the formulated diet to satiation. A total of 96 display tests always involving new combinations of individuals were conducted, and all individuals were tested six times, thus achieving an individual summated hiding score ranging from 0 to 6. A GLMM with a Poisson distribution of errors was used to compare the relationships between fork length and the sensitivity to natural predator between the two strains, using tank replicate as random factor.

Vulnerability to angling gear

On 26–31 August 2011, fishing experiments were conducted using the same fishing gear already used in the natural streams, which involved a 1.5 m pole (without a reel), a 1 lb line, a 0.5 g sinker equipped with a barbed hook (gape widths of 2.0 mm) equipped with a piece of frozen shrimp (about 5 mm total length) as natural bait. Five to six individuals of either the HP or the LP stream fish were randomly netted from the holding tank and transferred to an experiment tank ($W \times L \times D$: 32.6 cm × 45.7 cm × 30 cm) lacking cover. The tank was set up on a table 100 cm above the floor to avoid fish seeing the experimental angler. Fishing time of each trial was 15 min. Captured fish were checked for individual marks and immediately released back into the fishing tank to maintain an equal density. After each experiment, fish were transferred back into the holding tank and given the formulated diet to satiation. A total of 32 fishing experiments were conducted. Each individual was tested twice in a group, and the vulnerability score thus ranged from 0 to a maximum of 2 captures. A GLMM with a Poisson distribution of errors was used to compare the relationships between fork length and the vulnerability to angling between the two strains, using tank replicate as random factor. On 9 September 2011, fork length and body mass were again remeasured to serve as a measure of growth during the experimental phase.

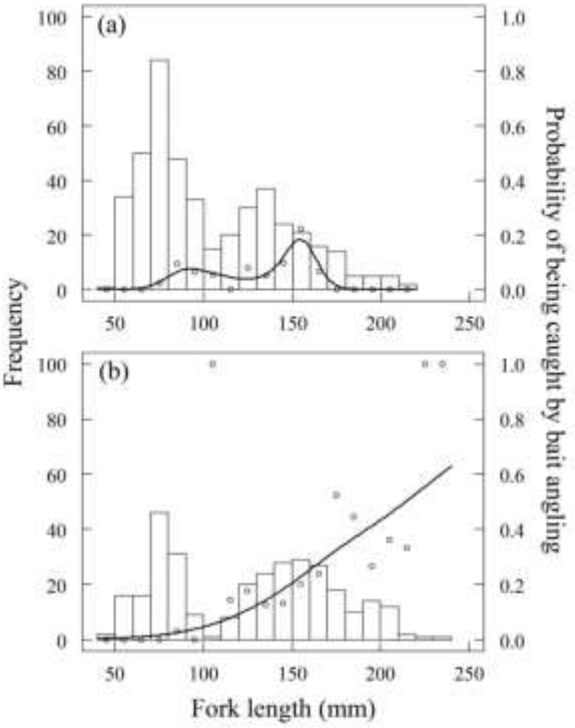
A GLMM with a normal distribution of errors was used to compare the relationships between fork length and the SGR during predator display and angling tests (from 16 August to 9 September 2011) between the two strains, using tank replicate as random factor. A generalized additive mixed model (GAMM; Zuur et al. 2009) was also used to assess possible nonlinear effects of fork length on SGR. SGR of the fishes during behavioural and angling experiments was separately analysed to test for differences in food ingestion rates and growth as a consequence of handling stress to which shy and bold individuals might react differently.

All analyses were conducted using the R package "mgcv" (version 1.7-29). The Akaike information criterion (AIC) was used to identify the best-fitting models.

Results

A total of 73 individual fish were captured by angling for 31 angler-hours, and 974 fish were caught by electrofishing (Table 2). Fork length of these fish ranged from 45 to 236 mm. Amago salmon captured in the HP stream were significantly smaller (HP: 110 ± 42.5 mm; LP: 121 ± 44.5 mm, mean fork length ± SD, $F = 17.64$, $p < 0.001$) and younger (HP: 0.61 ± 0.74; LP: 0.84 ± 0.88, mean years of age ± SD, $F = 19.11$, $p < 0.001$) than those in the LP stream (Fig. 2; also see online supplementary data Fig. S1[1]). Population densities did not differ among the two streams and ranged from 0.087 to 0.275 individuals per square metre (Mann–Whitney U test, $p = 0.602$; Table 2). Despite similar densities, the catch per unit effort (number of fish captured per angler-hour) of bait fishing in the HP stream (0.50–0.93–1.43, min.–mean–max.) was significantly lower than that in the LP stream (0.75–2.13–4.17, min.–mean–max.; Mann–Whitney U test, $p = 0.030$; Table 2). Both fork length and age were within the same range for fish caught by bait angling and electrofishing in the HP stream, but fish caught by angling were larger and older than those caught by electrofishing in the LP stream (Table 3). Fork length, stream, the interactions of fork length and age, and that of fork length and stream were significant factors affecting the vulnerability to bait angling (Table 4). Based on the AIC, a GAM fitted the data on vulnerability to angling better than a GLM (Table 4). The GAM showed that the vulnerability to angling increased with fork length in the LP stream, whereas the vulnerability to angling of fish from the HP stream initially increased with fork length and subsequently decreased with further increases in body size (Fig. 2). The significant interaction of fork length and age indicated that older fish were less vulnerable to angling, even at similar fork lengths (Table 4; Fig. S1[1]).

Fig. 2. Fork length distributions (left-side axis, histograms) and probability of being caught by bait angling (right-side axis, plots and spline curves) on amago salmon in a (*a*) high and (*b*) low fishing pressure stream.



In the laboratory experiments, there was no difference in SGR over a period of 36 days among the two strains before the predator display experiments were conducted (HP: 0.63 ± 0.28; LP: 0.56 ± 0.29, mean ± SD; Table 5). However, in the predator display experiments, the number of times fish were hiding under cover was significantly greater for HP fish (HP: 3.63 ± 1.42; LP: 2.86 ± 1.46 times, mean ± SD; Table 5; Fig. 3), indicating a greater intrinsic timidity of HP fish relative to LP fish. In contrast with the field data reported above for the juvenile and adult fish, under controlled conditions in the laboratory the number of captures of HP and LP fish was not significantly different for juveniles (Table 5). However, during the predator display and angling experiments in the laboratory, the relationship between the SGR assessed over 24 days and fork length was starkly different among the HP and LP fish (Table 5). A GAMM fitted the SGR data better than a GLMM for the period during the predator display and fishing experiments (Table 5). Accordingly, HP fish exhibited significantly lower growth rates than LP fish during the experiments, particularly the larger ones (Fig. 4). We repeated the analysis for fish <95 mm given the small sample sizes presented for the largest fish size classes (see Fig. 4). Even after removing these data, HP fish still showed a significantly lower SGR than LP fish during the predator display and angling experiments (Table 5).

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[1] Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2014-0571.

Table 3. Comparison of fork length (mm) and age (years) (mean ± SD) of amago salmon caught by angling and electrofishing in a high fishing pressure (HP) and low fishing pressure (LP) stream.

			Salmon caught by:			
Stream	Date of bait angling		Angling	Electrofishing	*F*	*p*
HP	8 Aug. 2008	Fork length	115±34	110±42	0.263	0.608
		Age	0.47±0.52	0.53±0.72	0.123	0.726
	27 June 2009	Fork length	128±31	99±35	4.532	0.034
		Age	0.71±0.49	0.48±0.56	1.254	0.264
LP	7 and 10 Aug. 2008	Fork length	164±27	110±40	54.945	<0.001
		Age	1.40±0.60	0.62±0.73	31.484	<0.001
	26 June 2009	Fork length	172±36	124±47	16.512	<0.001
		Age	2.25±0.93	0.90±0.93	30.820	<0.001

Table 4. The best model of a generalized linear model (GLM) and a generalized additive model (GAM) selected by Akaike information criteria (AIC) in a field experiment on the vulnerability to angling of amago salmon in a high and low fishing pressure stream.

Method	Error distribution	AIC	ΔAIC	Independent variable	Coefficient	*p*
GLM	Binomial	395.9	1.7	Stream	1.895	0.024
				Fork length	0.047	<0.001
				Age	2.733	0.011
				Stream × Age	−2.889	<0.001
				Fork length × Age	−0.016	0.007
GAM	Binomial	393.8	3.8	Stream	1.376	0.036
				s(Fork length)*	—	<0.001
				Stream × Age	−2.430	<0.001

Note: The independent variables were stream (high fishing pressure stream = 1, low fishing pressure stream = 0), fork length, age, and their interaction. ΔAIC shows the difference of AIC between the best and full models.

*p value shows approximate significance of smooth term (s).

Table 5. The best model of a generalized linear mixed model (GLMM) and a generalized additive mixed model (GAMM) selected by Akaike information criteria (AIC) for the laboratory behavioural and growth assays on age-0 year amago salmon originating from high and low fishing pressure streams.

Dependent variable	Method	Error distribution	AIC	ΔAIC	Independent variable	Coefficient	*p*
SGR before experiments	GLMM	Normal	48.0	14.9	Fork length	1.895	<0.001
No. of times hiding under cover	GLMM	Poisson	138.1	2.0	Strain	0.247	0.004
					Fork length	0.009	0.002
No. of times caught by bait angling	GLMM	Poisson	118.3	1.6	Fork length	0.032	<0.001
SGR during experiments using full data	GLMM	Normal	−36.2	0.0	Strain	0.705	0.066
					Fork length	0.016	<0.001
					Strain × Fork length	−0.014	<0.001
	GAMM	Normal	−96.8	0.0	Strain	0.643	<0.001
					s(Fork length)*	—	<0.001
					Strain × Fork length	−0.013	<0.001
SGR during experiments using data of <95 mm fish	GLMM	Normal	−74.1	4.7	Strain	−0.262	0.013
					Fork length	0.007	<0.001

Note: The independent variables were strain (originating from high fishing pressure stream = 1, low fishing pressure stream = 0), fork length at the start of each duration, and their interaction. ΔAIC shows the difference of AIC between the best and full models. SGR = specific growth rate.

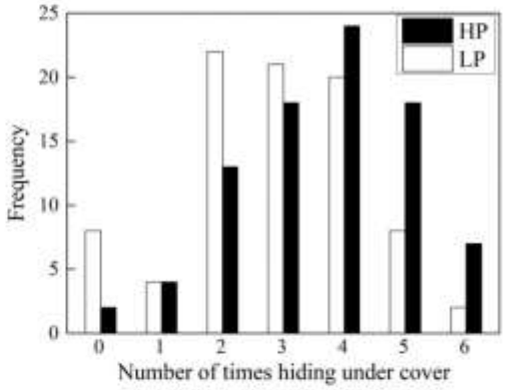
*p value shows approximate significance of smooth term (s).

Discussion

We found large amago salmon to be more vulnerable to angling in an LP stream compared with their smaller conspecifics, while no generally positive size selectivity was present in a HP stream. Moreover, the catch rates of amago salmon were significantly lower in the HP stream, despite similar fish abundances, indicating a substantial reduction of vulnerability to angling, particularly among the large fishes. Such patterns were in agreement with previous research in selected recreationally targeted coastal fish species (Alós et al. 2015). We also provided evidence that fish from the HP stream were shyer and more risk-averse compared with fish from the LP stream, which agreed with laboratory-based harvesting experiments using zebrafish (*Danio rerio*) (Uusi-Heikkilä et al. 2015). Recent studies in brown trout (*Salmo trutta*) and Eurasian perch (*Perca fluviatilis*) found that explorative individuals were preferentially hooked by anglers (Härkönen et al. 2014, 2015). We thus propose that in the HP stream, behaviourally reactive individuals (e.g., active and (or) bold fishes) have been systematically removed from the pool of fishes, leaving behind shyer individuals that are harder to catch.

Given the common garden nature of our experiments using fry that have not been exposed to any angling in the wild, our data may be perceived consistent with an evolutionary response towards increased shyness in response to selective removal of bold fish in the HP stream. However, in the field additional effects of learning to avoid future capture were likely involved, because

Fig. 3. Frequency distributions of the number of times hiding under cover in the predator display experiments on age-0 year amago salmon originating from a high fishing pressure (HP) and low fishing pressure (LP) stream.

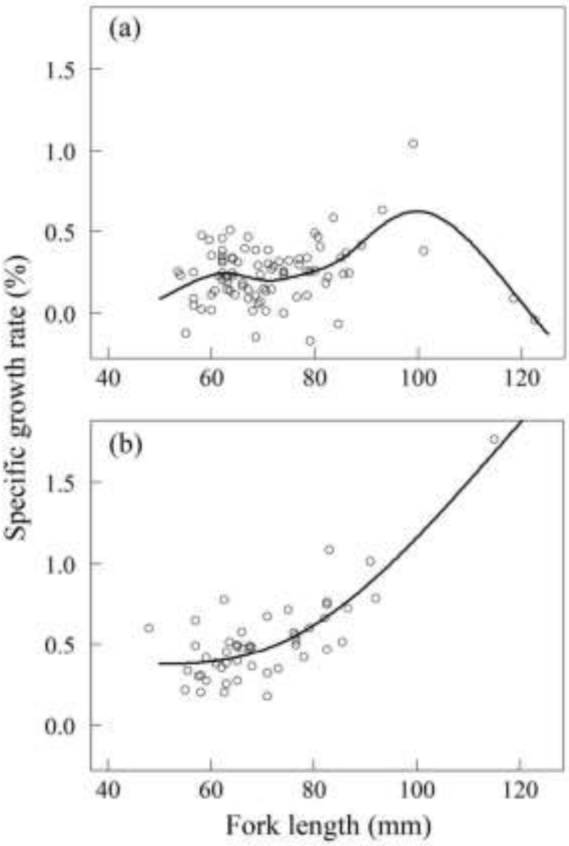


there was no intrinsic difference in angling vulnerability of amago fry under controlled laboratory conditions, and vulnerability in the field decreased further as fish aged, suggesting experiential learning. Indeed, rainbow trout (*O. mykiss*) exposed to catch-and-release angling altered their behaviour and became more wary, in turn substantially reducing catch rates due to hook-avoidance learning (van Poorten and Post 2005; Askey et al. 2006). Moreover, a major limitation of our study is the lack of stream replication, and hence we cannot conclusively relate our study findings to fishing pressure in a cause-and-effect or evolutionary manner. Although spatial proximity and general ecological conditions (including density) were similar among the streams we studied (Tsuboi et al. 2013) with only fishing pressure sticking out as a discriminatory feature, unaccounted ecological factors could have contributed to the study findings. Further research with more streams is needed to confirm the data and the interpretation presented in this paper, but such systems do not exist in our study area. Irrespective of the exact mechanism, any fisheries-induced adaptive response to reduce exposure to fishing gear will not only reduce CPUE and hence angling quality (Arlinghaus 2006; Arlinghaus et al. 2008*a*), but may even obscure the often-reported effect of positive size selectivity of recreational fishing as found here. As a consequence, angler-exploited populations could host large fish that are invulnerable to angling, in turn promoting increasingly dome-shaped selectivity curves that have been repeatedly reported for hook-and-line gear (e.g., O'Farrell and Botsford 2006; Hutchings 2009).

Superficially, the lack of differences of angling vulnerability between the common garden raised fry from HP and LP streams in the laboratory environment may be interpreted as being inconsistent with an evolved response of low vulnerability to fishing. However, an emerging literature is questioning the validity of certain laboratory studies and test areas for revealing complex behaviours and fish personality (Klefoth et al. 2012; Niemelä and Dingemanse 2014), including work in salmonids (Näslund et al. 2015). Similarly, Wilson et al. (2011) failed to find a clear relationship of boldness and vulnerability to angling in a sample of seined bluegill (*Lepomis macrochirus*) when tested in a laboratory environment. The lack of vulnerability differences between HP and LP fish in the laboratory may have been caused by the close proximity to the bait in a tank lacking shelter. By contrast, several studies conducted under seminatural or even natural conditions have now revealed unambiguous relationships of boldness (or correlated traits such as exploration, activity, or choice of risky pelagic habitats) and vulnerability to angling in a range of species (Klefoth et al. 2012, 2013; Matthias et al. 2014; Härkönen et al. 2015; for a theoretical model see also Alós et al. 2012). While we failed to identify vulnerability differences among HP and LP fish in the laboratory, we found age-0 HP fish to avoid the simulated natural predator significantly more frequently than age-0 LP fish, and these fish also grew less under risk of predation under laboratory conditions. Coupled with the field data on differences in vulnerability, we cautiously interpret our data as consistent with an evolved boldness response, leading to greater shyness and lower vulnerability to fishing in highly exploited streams.

Recreational fishing mortality can be very high in some localities and strongly affect fish populations demographically (Post et al. 2002; Lewin et al. 2006). In particular, heavy catch-and-kill type angling is known to substantially alter age and size distributions within salmonid and other fish populations (e.g., Goedde and Coble 1981; Braña et al. 1992). Size and age truncation was also present in our work where we observed differences in fork length and age among HP and LP streams. In fact, although the population in the exploited HP stream was not numerically less abundant (similar to the coastal fisheries case reported by Alós et al. 2015), the HP population hosted overall smaller and younger fishes compared with the population in the LP stream. Amago salmon in HP streams are traditionally removed after capture, and hence the stock composition in terms of a truncated size and age distribution was not unexpected. The lower vulnerability of large, fecund individuals expressed in HP environments may also safe-

Fig. 4. Relationships between specific growth rates and fork length during predator display and angling tests in tanks on age-0 year amago salmon originating from a (*a*) high and (*b*) low fishing pressure stream.



guard against population collapses. To conclude, fishing-induced adaptive changes, be it evolution or plasticity or both, towards lower vulnerability are likely to carry costs for fishing quality and the index quality of fishery-dependent data while safeguarding population viability in the face of exploitation. However, the presence of more shy individuals in heavily exploited system may have undesired food web effects and could alter ecosystem function (Laundré et al. 2014), which should be studied in the future.

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Paper VI

VI

Thomas Klefoth, Tonio Pieterek, Robert Arlinghaus, 2013. Impacts of domestication on angling vulnerability of common carp, *Cyprinus carpio*: the role of learning, foraging behaviour and food preferences. Fisheries Management and Ecology, 20:174-186.

Fisheries Management
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Impacts of domestication on angling vulnerability of common carp, *Cyprinus carpio*: the role of learning, foraging behaviour and food preferences

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Abstract Domestication in fish selection increases vulnerability to angling. Two common garden-reared genotypes of common carp, *Cyprinus carpio* L., differing in degree of domestication (highly domesticated mirror carp and less domesticated scaled carp) were exposed to fishing in two environments (i.e. ponds and laboratory tanks) to quantify vulnerability to angling. Foraging behaviour and food preferences were quantified to explain variation in angling vulnerability in a mechanistic manner. Domesticated mirror carp were more vulnerable to angling gear than scaled carp in both environments, which was related to greater food intake and bolder-foraging behaviour. Independent of genotype, catchability decreased and time until first capture increased over fishing time, indicating learned hook avoidance. No differences were observed in food preferences among genotypes, rendering bait-selective feeding an unlikely explanation for differential vulnerability to angling. It was concluded that vulnerability to angling has a genetic basis in carp and that boldness plays a paramount role in explaining why more domesticated genotypes are more easily captured by angling.

KEYWORDS: adaptation, catchability, common garden, fisheries-induced evolution, hook avoidance, social learning.

Introduction

Predator–prey relationships like those between anglers and their target fish are regulated by the fish's antipredator behaviours (Cox & Walters 2002; Van Poorten & Post 2005; Askey *et al.* 2006). Antipredator behaviour in fish has a genetic basis (Seghers 1974; O'Steen *et al.* 2002; Ghalambor *et al.* 2004), but experience and learning over time can strongly influence the development of antipredator responses (Kieffer & Colgan 1992; Kelley & Magurran 2003; Griffin 2004). The propensity of fish to learn from experience has been found to be higher in fish adapted to high-predation environments than fish adapted to low-predation environments (Huntingford & Wright 1992), indicating that not only antipredator behaviour *per se*, but also the ability to learn, has an evolutionary basis. Therefore, a different degree of adaptation to natural predation risk might also influence

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responses of fish towards baited hooks in recreational fisheries.

Management of freshwater recreational fisheries often involves stocking activities using fish raised in captivity (Pearsons & Hopley 1999; Arlinghaus *et al.* 2002). Therefore, individual fish of the same species and within the same water body can substantially differ in their local adaptation towards predation risk, because phenotypic traits related to risk-taking are often altered by the domestication process within fish hatcheries in ecological or evolutionary contexts (Price 1999; Huntingford 2004; Sundström *et al.* 2004). The combination of selective breeding and early life experiences of unnaturally low-risk conditions within culture facilities strongly shapes fish behaviour (Price 1999). As a consequence of the domestication process, cultured fish can be assumed to represent the risk-taking end of the behavioural spectrum (Huntingford 2004). Fish raised under culture conditions are thus usually more vulnerable towards angling than their wild conspecifics as has been shown for a variety of salmonids [brook char, *Salvelinus fontinalis* (Mitchill) (Flick & Webster 1962); cutthroat trout, *Oncorhynchus clarkii* (Richardson) (Dwyer 1990); rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Dwyer & Piper 1984); brown trout, *Salmo trutta* L. (Mezzera & Largiadèr 2001)]. Similarly, differences in angling vulnerability between high- and low-domesticated genotypes of omnivorous common carp, *Cyprinus carpio* L., have been reported where domesticated mirror carp were more readily captured by passive angling tactics than their less domesticated, scaled conspecifics (Beukema 1969; Raat 1985). However, little is known about the specific traits that render more domesticated genotypes more vulnerable to angling, although boldness behaviour is likely to play an important role (Klefoth *et al.* 2012). In addition, preferences for artificial food (such as purposely made baits or corn kernels in carp fishing, Niesar *et al.* 2004; Rapp *et al.* 2008) or ability to digest carbohydrate-rich cereals, to which domesticated fish have been ecologically or evolutionarily adapting under farm conditions, may play an important role (Suzuki *et al.* 1978).

In general, the impact of fish learning abilities on angling catch rates has been shown for different predatory species by documenting declining catch rates with increasing fishing pressure in a catch-and-release fishing context [largemouth bass, *Micropterus salmoides* Lacepède (Anderson & Heman 1969; Hackney & Linkous 1978); rainbow trout (Van Poorten & Post 2005; Askey *et al.* 2006); northern pike, *Esox lucius* L. (Beukema 1970a; Kuparinen *et al.* 2010)]. Similarly, omnivorous carp were found to learn from previous capture events insofar as fish that were already captured within a fishing season were found less likely to be caught in the future compared with previously uncaptured conspecifics (Beukema 1970b; Raat 1985). However, it remains unclear whether differently domesticated fish differ in their ability to avoid being hooked as a consequence of learning. Four issues that are not mutually exclusive are worth noting. First, as domesticated fish might be selected for rapid growth, there might be a corresponding selection for reduced allocation of energy into the building of the neural network leading to reduced cognitive abilities (Mery & Kawecki 2003). Second, the holding of domesticated fish in artificial conditions might reduce the ability of these fish to learn complex tasks (Marchetti & Nevitt 2003). For these two reasons, domesticated fish might express a constantly higher catchability over time relative to less domesticated fish. An alternative perspective is that cultured fish might be initially more vulnerable to angling than less domesticated individuals, but, with increasing experience, differences between the two groups might vanish owing to social learning effects (Beukema 1970b; Krause 1993; Brown & Laland 2003). A final issue is that differences in vulnerability between fish of high and low domestication (and hence adaptation to natural predation risk) might be strongly dependent on the ecological context as a consequence of genotype × environment interactions (Klefoth *et al.* 2012). Therefore, differences in vulnerability among high- and low-domesticated genotypes might be context dependent.

Another reason for differences in angling vulnerability between fish with differences in their adaptation to culture conditions could be related to food preferences, consumption rate and food-intake speed. Experiments determining differences in the feeding behaviour of wild and domesticated brown trout from the same river showed that wild trout ate more and were quicker to attack and consume prey than domesticated trout (Sundström & Johnsson 2002). Similarly, using differently domesticated carp strains reared in a common garden, Matsuzaki *et al.* (2009) found wild carp to attack prey items more rapidly and to have higher consumption rates than highly domesticated genotypes. However, both studies were based on natural food items. Suzuki *et al.* (1978) used commercial fish pellets and natural worms in trials with carp and found domesticated carp to prefer artificial food over natural food resources and to be more readily captured on the non-natural food. Translating these results into an angling context using baits like worms or maggots, wild genotypes should be more vulnerable to angling than their domesticated counterparts. However, in most contemporary angling for carp, artificial bait items are used such as corn kernels, other seeds or so-called boilies (Niesar *et al.* 2004). Domesticated carp might show a higher consumption rate of this artificial food, which might be one mechanism for the higher vulnerability of these genotypes using such baits (Suzuki *et al.* 1978).

The objective of this study was to analyse the vulnerability to angling over time and its mechanistic basis as revealed by two genotypes of common garden reared carp (scaled and mirror) with known differences in genetic adaptation to low-risk culture conditions. Tests for angling vulnerability were conducted during day and night in two different environments (i.e. laboratory tanks and ponds) to investigate the influence of the environment and visual cues on threat recognition and learned hook avoidance. Laboratory experiments on foraging activity and preferences of a novel food item were conducted in a two-way-choice experiment to understand some of the mechanisms responsible for differences in vulnerability between the two genotypes of carp. It was hypothesised that more domesticated carp would be more vulnerable overall, and vulnerability would shift to the dark periods with increasing fishing pressure. It was also hypothesised that reasons for the differential vulnerability would be related to increasing consumption rates and a preference for corn kernels as bait among domesticated carp. Finally, wild genotypes were assumed to exhibit greater learning to avoid future capture compared with domesticated carp.

Material and methods

Study animals

Study animals involved two genotypes of common carp differing by distinct scale patterns reflecting its evolutionary history of adaptation to aquaculture conditions as described by Klefoth *et al.* (2012). All carp used were raised at a commercial fish hatchery (Fischzucht Wegert, Ostercappeln, Germany, 52°19′52″ N, 8°14′48″ E) in the same common garden pond environment. Parental fish descended from two selection lines: (1) a selection line with scaled morphotypes; and (2) a selection line in which scaled morphotypes were previously crossed with strongly domesticated mirror carp selection lines. Fish from both selection lines were stocked into the same common garden pond for reproduction. Young-of-the-year mirror carp could only develop as a result of two breeders from the strain originally crossed with domesticated mirror carp (strain 2) (Kirpichnikov & Billard 1999). All juvenile carp were exclusively fed with standard carp dry food in addition to any natural food ingested in the shallow (1.5 m deep) earthen common garden pond (40 m × 50 m). At an age of 10 months, a subsample of the fish was transported to the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin, Germany, for angling experiments, and another subsample was transported to a laboratory at Humboldt-Universität zu Berlin, Germany, for laboratory tests on feeding behaviour and food preferences of the fish. Juvenile carp were chosen as experimental fish because use of much larger and older fish would have been impractical under laboratory conditions. In both locations, fish were kept in tanks (1 m × 1 m × 1 m; 5 fish per 100 L) with tap water in a recirculation system (mean temperature ± SD 18 ± 1.5 °C, exchange rate about once per day). Fish were fed with standard carp pellets (5 mm diameter; Aller Classic, Aller Aqua, Golßen, Germany), and the total daily food amount was approximately 1.5% of fish body wet mass.

Tagging of fish

All carp ($N = 100$ scaled carp and $N = 100$ mirror carp used for angling experiments and $N = 54$ scaled carp and $N = 54$ mirror carp used for laboratory experiments on foraging activity and food preferences) were individually marked with passive integrated transponders (PIT) for individual identification. PIT (23 mm length, 2 mm width; Oregon RFID, Portland OR, USA, 2% tagging mortality) were surgically implanted into the fish's body cavity following the method described by Skov *et al.* (2005). Before PIT implantation, fish were anaesthetised using a 1 mL L^{-1} of 9:1 solution of ethanol/clove oil in well-aerated water at 18 °C. After PIT implantation, all fish were measured for total length (TL, to nearest mm) and allowed to recover for a minimum of 3 days before experiments started.

Tests for angling vulnerability within ponds

To investigate the vulnerability to angling of scaled and mirror carp under seminatural conditions in ponds, each of three pond replicates (12 m × 5 m × 1 m; L × W × H) was stocked with 40 similar-sized carp (20 scaled carp and 20 mirror carp, mean TL ± SD pond 1: 199 ± 6.9 and 199 ± 12.1 mm, *t*-test, $t = 0.08$, $P = 0.936$; pond 2: 199 ± 4.7 and 200 ± 11.9 mm, *t*-test, $t = -0.26$, $P = 0.797$; pond 3: 199 ± 6.1 and 197 ± 11.2 mm, *t*-test, $t = 0.78$, $P = 0.440$). Fish were allowed to acclimatise for 9 days before angling experiments started. Pond experiments were conducted in September 2008. The ponds were continuously supplied with water from the nearby Müggelsee in Berlin (800 ha; shallow; eutrophic). Inflow into the ponds was about 1 L s^{-1} unfiltered lake water. The environmental conditions in the ponds were documented using temperature loggers (TidbiT datalogger; Onset, Bourne, MA, USA). Mean water temperature ± SD in the ponds over the study period was 16.2 ± 1.9 °C (range: 13.9–19.5 °C). The ponds contained a shelter structure (2 m × 5 m) made out of black plastic material just above the water surface next to the water inlet, and two pre-defined round angling locations (diameter 0.5 m) at different distances to the shelter (3 and 7 m away from the shelter structure, respectively). Angling was conducted for 20 consecutive days, simultaneously in all three ponds and started 2 h before sunset until 2 h after sunset (total daily angling effort per pond = 4 h). The starting time of daily angling events was alternated every day among the morning or evening twilight periods, and fishing always involved daytime and nighttime periods to estimate the impact of diel period on angling vulnerability. Angling was conducted on the pre-defined angling locations and position of hook placement was alternated between the two locations on a 60-min basis to control for potential feeding location preferences by the study fish. In addition to the baited hook, standard carp pellets (5 mm diameter) were used as pre-baiting food and were provided every 60 min on the feeding locations. Total daily food amount of pre-baiting pellets per pond was 1% of fish body wet mass at the time of stocking (approximately 12 pellets). In addition, for every single pellet, one sweet corn (5–7 mm diameter; Bonduelle, Reutlingen, Germany) was offered, which also served as carp bait on the hook.

Angling and feeding took place simultaneously on the same locations. Sweet corn kernels were used as bait, provided on a bold-rig as described by Rapp *et al.* (2008). This method ensured exclusive shallow hooking of the fish. The hook was connected to a 13-cm multifilament soft leader. The angling equipment consisted of a 3-kg monofilament line, a 15-g sinker, and a short fishing rod (2.1 m, 0.3 lb test curve; Balzer, Lauterbach, Germany). Bites were indicated by an electronic bite indicator (Carp-Sounder Basic VR; Carp-sounder, Germany). After potential self-hooking, the fish was landed quickly using a rubber-net to prevent mucus abrasion (Barthel *et al.* 2003). Fish were then placed into a bucket filled with fresh water for unhooking and PIT identification (Pocket reader; Allflex, Dallas, TX, USA). Afterwards, fish were immediately released in the middle between the two feeding locations. Release of the fish was always conducted within 30 s and no mortality occurred. In addition to the individual ID of captured fish, time of every bite as indicated by the bite indicator was recorded to the nearest 10 s.

Tests for angling vulnerability within laboratory tanks

Additional laboratory-based experiments under controlled environmental conditions were conducted to test for the consistency of differences in angling vulnerability among the two carp genotypes under fully controlled environmental conditions. This experiment resembled the set-up established in the ponds and used a new set of study animals. This was done to avoid experimental biases based on learning effects of the fish that might have occurred if individuals from the pond environment would have been re-used during laboratory experiments. Experiments were conducted in a large laboratory tank (10 m × 4 m × 1 m; L × W × H) of comparable size with the ponds. The tank was connected to a circulating water system and a biological filter. Water inflow was $2\ \mathrm{L\ s^{-1}}$ and water temperature ± SD was constant at 22 ± 1 °C. To investigate vulnerability in the laboratory, a total of 40 similar-sized fish (20 scaled carp and 20 mirror carp, mean TL ± SD 225 ± 20.0 and 229 ± 16.0 mm, respectively, *t*-test, $t = -0.80$, $P = 0.441$) was stocked into the tank and allowed to acclimatise for 9 days before angling experiments started. Laboratory experiments were conducted in March to April 2009. The experiments followed the same protocol described for the pond experiment, with the exception that intervals of changing the fishing locations within the daily 4 h angling periods were 15 min instead of 60 min for logistical reasons. After the 7th day of angling in the laboratory environment, experimental carp were replaced by a new set of 40 fish (20 scaled carp and 20 mirror carp, mean TL ± SD 224 ± 17.0 and 229 ± 21.0 mm, respectively, *t*-test, $t = -0.89$, $P = 0.377$), and the experiment was replicated.

Foraging behaviour and food preferences of scaled and mirror carp in the laboratory

Behavioural laboratory experiments were conducted in 12 different aquaria of similar size (100 × 40 × 40 cm) to test for differences in feeding behaviour and food preferences between scaled and mirror carp as possible mechanistic explanations for differences in angling vulnerability between the two genotypes. Aquaria were positioned along two walls using metal racks (three tanks abreast and two tanks stacked) in different distances to a window (2 and 4 m). All aquaria were connected to an independent circular filtering system along each wall. Each tank was equipped with a flat metal rail placed at the bottom to divide aquaria visually into a similar-sized left and right compartment, without influencing activities of fish swimming between the two compartments. Focal fish (mean TL ± SD of scaled carp 230 ± 25 mm and mirror carp 237 ± 30 mm, *t*-test, $t = 1.1$, $P = 0.35$) consisted of individuals from the same common garden as those individuals used for angling experiments. Individuals were randomly assigned to one of the aquaria and allowed to acclimatise for 7 days. Water temperature within the aquaria was 19.7 ± 1.2 °C. During acclimatisation, fish were fed pellets (same as previously) with an amount of approximately 1% of body wet mass per day, except for the day before observations started, and aquaria were cleaned on a daily basis to remove residual food. Because no food was provided on the last day before the experiments, fish were starved for about 17 h before behavioural observations started.

Focal fish were tested individually for their feeding behaviour and their food preferences for pellet vs sweet corn kernels following an observer-based approach where the observer stood about 3 m away from the aquaria to observe directly behaviour of the fish. During experimentation, the observer provided five items of two different food sources to the fish (five pellets and five corn kernels). Food items (pellets and corn kernels) were similar to those used during angling experiments. The test procedure for food preferences of scaled and mirror carp followed a two-way-choice setup (Holbrook & Schmitt 1988) where the fish had to choose between two different sources of food provided in different areas of the aquaria at the same time. Behavioural variables evaluated were the number of side changes within 10 min after food supply as an indicator of activity during feeding and the time elapsed until first ingestion of a food item. Afterwards, the number of pellets and corn kernels eaten within 10 min and the total sum of food items eaten (maximal 10 food items per replicate) were counted. Behavioural data were replicated three times, and tests for food preferences were replicated six times. Afterwards, the fish in the aquaria were replaced by a new set of focal fish.

Effects of genotype, angling duration and diel period on catchability of carp

Generalised linear mixed models were used to explain the daily absolute number of angling captures within ponds and laboratory tanks. The data set was used to test for differences in the number of captures between scaled and mirror carp (Genotype), the impact of angling duration on captures measured as consecutive fishing days of the experiment (Day) and the diel period at capture on each specific fishing day, distinguishing day and night (Diel period). Diel period was defined as either daytime (period from sunrise to sunset) or nighttime (period between sunset and sunrise). All possible two-way and three-way interactions with Genotype, Day and Diel period were added to the models and subsequently removed if not significant at $P < 0.05$. Pond and tank replicates were added as a random factor to account for inter-replicate variability. In addition, mean daily water temperature was added as a random factor to the model explaining number of angling captures in the pond environment. In all cases, data were not overdispersed and a Poisson error distribution was found to be the best fit to the data. The software package R and function lmer in library lme4 (R Development Core Team 2009) was used for analysis. Variances explained by the models were calculated using Nagelkerke's Pseudo-R^2.

To investigate the potential for learning ability of the fish as a response towards angling pressure, time until the first capture of the day was regressed on the time until the first bite of the day and on angling duration (defined as the consecutive fishing day of the experiment) using linear regression models. Variance components of pond and tank replicates were low (<1%), indicating minor differences between replicates in the time until the first capture of the day, so that the regressions were calculated using mean values of pond and tank replicates per day. Interaction terms were removed if not significant. One would generally expect the time until first bite and time until first capture of a fish to be positively related. Learning would be indicated by a positive relationship between time until first bite or time until first capture and fishing duration. Moreover, if over time a decoupling of the time elapsed until the first bite and time elapsed until the first capture happens, this would become visible as a non-significant correlation of these variables indicative of learned hooking avoidance over time.

Mechanistic explanations for differences in vulnerability between scaled and mirror carp

Differences in swimming activity during foraging, time to ingest the first food item and the total amount of food items eaten between scaled and mirror carp were tested using linear mixed models. General foraging activity is known to be correlated with boldness, and boldness, measured as foraging activity, differs among mirror and scaled carp (Klefoth *et al.* 2012). Also, genotypes with shorter time to ingest food and greater total food consumption would have a greater likelihood of capture. The dependent variables swimming activity (measured by the number of side changes of the fish while feeding), time to ingest the first food item (measured in seconds) and the total amount of food items eaten were log-transformed to reach normality and homogeneity of variances prior to analyses. The repeated measures design of the experiment (six observations per individual) was accounted for using individual ID as a random factor. Further, position of the aquaria within the wet-laboratory (i.e. close or distant to a window) was added as a random variable. Genotype was added as a fixed factor and body size (TL) of the fish was added as a covariate in all models. The interaction of the two fixed predictor variables was insignificant in all cases and deleted for final analyses.

Food preferences of scaled and mirror carp were analysed using paired *t*-tests. The total number of pellets and corn kernels ingested during the experiment was separately compared for both genotypes. Using this approach, differences in food preferences between scaled and mirror carp would become obvious if the paired *t*-tests would indicate a significant preference of one sort of food for one genotype, but not for the other. To quantify the explanatory power of the results, effect sizes d for paired *t*-tests were calculated following the approach outlined in Gliner *et al.* (2001) and interpreted as weak ($d \geq 0.2$), medium ($d \geq 0.5$) or strong ($d \geq 0.8$) effects following Cohen (1988).

Results

Effects of genotype, angling duration and diel period on catchability of carp

Within all ponds a total of 19 scaled carp (32%) and 33 mirror carp (55%) were captured over a period of 20 angling days. One individual scaled carp was captured three times, whereas no other scaled carp was captured more than once. Out of the vulnerable pool of mirror carp, eight individuals were caught twice, one individual was captured three times and another individual was captured four times. The numbers of daily captures within the pond environment were significantly higher for mirror carp than scaled carp (Table 1), and the number of fish caught per day (expressed as a fraction of total stock size, i.e. catchability, in Fig. 1) generally decreased over the course of the experiment (Table 1; Fig. 1). Because interaction terms including genotype were insignificant in all models, this indicated a generally increasing hook avoidance with increasing fishing pressure and angling experience of the fish for both scaled and mirror carp. No effects of diel period on catchability of the fish were observed within ponds in neither of the two genotypes, as indicated by an insignificant Diel period × Genotype interaction (Table 1).

Table 1. Generalised linear mixed models to explain the variability of genotype-specific daily angling captures within three replicated ponds and two replicated laboratory tanks based on carp genotype, diel period and consecutive day of angling. Total angling period was 20 days (ponds) and 7 days (laboratory). The models assumed Poisson errors. Pond and laboratory replicates and mean daily water temperature within ponds were considered as random effects. Non-significant interactions were removed from the final models

Parameter	Estimate	SE	Z	P	R^2
Pond					
Intercept	−0.874	0.31	−2.86		0.29
Genotype (mirror)	0.784	0.26	2.98	**0.003**	
Diel period (night)	0.150	0.25	0.61	0.547	
Day of angling	−0.108	0.02	−4.57	**<0.001**	
Laboratory					
Intercept	1.867	0.30	6.27		0.69
Genotype (mirror)	0.664	0.21	3.19	**0.001**	
Diel period (night)	−1.353	0.39	−3.49	**<0.001**	
Day of angling	−0.535	0.09	−5.76	**<0.001**	
Diel period (night) × day of angling	0.449	0.12	3.84	**<0.001**	

Similar to the results in the ponds, mirror carp were significantly more vulnerable to capture by angling than their scaled conspecifics under controlled environmental conditions in the laboratory (Table 1). Here, 22 scaled carp (55%) were caught of which three individuals were caught twice, another three individuals were caught three times and a single scaled carp was caught six times. In comparison, 34 mirror carp (85%) were caught in the laboratory experiments, of which 14 individuals were caught twice, four individuals were caught three times and another four individuals were caught four times. The daily number of captures did not only significantly decrease with time spent fishing (Table 1, Fig. 1), but significantly more fish of both genotypes were captured during night, indicating effects of diel period on catchability of the fish within the laboratory. This effect was indicated by a significant Diel period × Day of angling interaction (Table 1; Fig. 2). The response towards increasing fishing time and time of the day, as measured by the number of daily capture events, did not differ between the two genotypes.

Within the pond environment, a significant positive association between time elapsed until the first bite of the day and time elapsed until first capture was found (Table 2; Fig. 3), and in addition, time until first capture was positively related to the day of fishing (Table 2; Fig. 3). This indicated a behavioural response of learned hook avoidance of carp towards increasing angling-induced risk. Even stronger effects of learned hook avoidance were found within the laboratory where the time elapsed until the first capture of the day was decoupled of the time elapsed until the first bite of the day (Table 2; Fig. 3), while a significant positive relationship between increasing fishing pressure and the time until the first capture of the day persisted (Table 2; Fig. 3). This indicated that fish remained actively foraging at the feeding locations at any time of the experiment, but with increasing fishing effort and duration carp more effectively avoided being hooked during their foraging

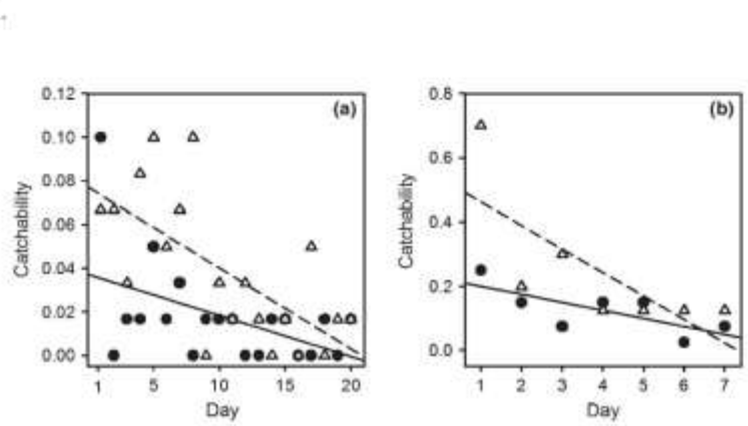

Figure 1. Relationship between the daily catchability (expressed as the total number of fish caught of a given genotype relative to all fish of that genotype) of scaled carp (filled circles, solid line) and mirror carp (triangles, dashed line) and the number of consecutive angling days within ponds (a) and laboratory tanks (b).

activities. This finding was additionally confirmed by visual observations (see Appendix S1 for a video showing active foraging and hook avoidance).

Mechanistic explanations for differences in vulnerability between scaled and mirror carp

Potential reasons for higher vulnerability of mirror carp were derived from the additional behavioural experiments in aquaria. Here, mirror carp were found to be significantly more active during foraging than scaled carp (mean number of side changes within aquaria ± SD of scaled carp 13.4 ± 2.1 and mirror carp 21.8 ± 1.7, respectively, linear mixed model, $\text{d.f.}_{\text{numerator}} = 1$, $\text{d.f.}_{\text{denominator}} = 107.9$, $F = 6.57$, $P = 0.012$). Mirror carp also ingested a higher total amount of food items during all six replicates, lasting 10 min each (mean total number of food items ± SD consumed by scaled carp 24.8 ± 1.2 and mirror carp 33.1 ± 1.4, respectively, linear mixed model, $\text{d.f.}_{\text{numerator}} = 1$, $\text{d.f.}_{\text{denominator}} = 104.9$, $F = 9.13$, $P = 0.003$), and they took significantly less time to ingest the first food item [mean time (s) to ingest the first food item ± SD of scaled carp 22.5 ± 7.0 s and mirror carp 7.2 ± 3.7 s, respectively, linear mixed model, $\text{d.f.}_{\text{numerator}} = 1$, $\text{d.f.}_{\text{denominator}} = 102.8$, $F = 7.2$, $P = 0.009$]. These behaviours likely contributed to why mirror carp were significantly more vulnerable to passive angling tactics than scaled carp in pond and large laboratory tank trials. However, no differences in food preferences between the two genotypes were found as indicated by similar and highly significant preferences for corn kernels over pellets by both genotypes during all six replicates lasting 10 min (mean total number of corn kernels and pellets consumed ± SD by scaled carp 19.3 ± 1.3 and 7.9 ± 0.8, respectively, paired *t*-test, d.f. = 53, $t = 9.2$, $P < 0.001$, $d > 0.8$; mean total number of corn kernels and pellets consumed by mirror carp 22.4 ± 1.4 and 12.7 ± 1.0, respectively, paired *t*-test, d.

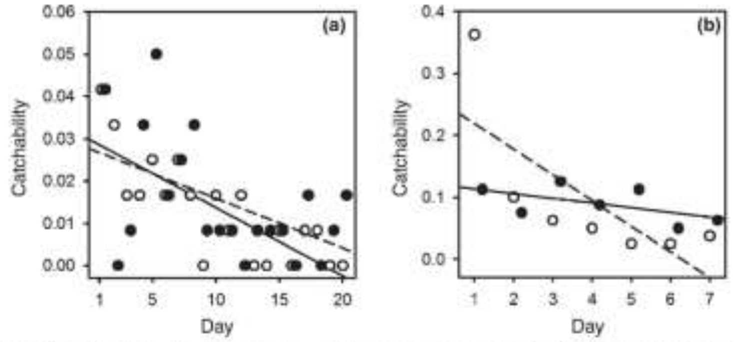

Figure 2. Daily catchability of carp (scaled and mirror carp grouped together in light of similar decline in catchability) during the day (white circles, dashed line) and at night (black circles, solid line) in relationship to the number of consecutive angling days within ponds (a) and laboratory tanks (b).

Table 2. Linear regressions to explain the time elapsed until the first angling capture of the day based on the time elapsed until the first bite of the day and the consecutive fishing day within ponds and laboratory tanks. Variance components for pond and tank replicates were very low (<1%), and therefore, analyses were conducted based on mean values of pond and tank replicates. The interaction terms were non-significant and removed from the final models

Parameter	Estimate	SE	t	P	R^2
Pond					
Intercept	22.634	14.89	1.52		0.80
Day of angling	3.953	1.48	2.67	**0.016**	
Time until first bite	0.924	0.17	5.49	**<0.001**	
Laboratory					
Intercept	−21.532	13.60	−1.58		0.87
Day of angling	18.928	3.17	5.98	**0.004**	
Time until first bite	0.380	0.65	0.59	0.590	

f. = 53, $t = 7.3$, $P < 0.001$, $d > 0.8$). These results indicate that corn kernels used as bait in the angling experiments was preferred as a food resource by both genotypes, and thus, the bait chosen in these angling experiments was not responsible for the differential vulnerability shown by scaled and mirror carp.

Discussion

Highly domesticated mirror carp were significantly more vulnerable to angling than their less domesticated scaled conspecifics in ponds, confirming the findings of previous studies (Beukema 1969; Raat 1985). In addition, the experiments showed that mirror carp were also more vulnerable to passive angling tactics within the laboratory under standardised environmental conditions, highlighting the consistency of differences in vulnerability between scaled and mirror carp. Because the two genotypes of carp used in this study were raised in a common garden, the results reveal a genetic basis of the composite trait angling vulnerability, as previously shown for other species (Dunham *et al.* 1986; Philipp *et al.* 2009). However, daily catch rates of the two genotypes decreased over the course of the study with increasing fishing pressure, suggesting active learning effects of carp to avoid being hooked as described by Beukema (1969, 1970b) and Raat (1985). Therefore, it is expected that differential vulnerability of scaled and mirror carp to capture is most strongly expressed early in the fishing season. Additional behavioural experiments in aquaria showed that potential reasons for higher vulnerability of mirror carp compared with scaled carp could be related to higher foraging activity, faster ingestion of food items and generally higher foraging rates of the mirror carp genotype. This agrees with greater boldness of the more domesticated carp genotype (Klefoth *et al.* 2012).

With increasing angling duration, the number of captures, independent of genotype, significantly declined in both the pond and the laboratory environment. This finding is most probably related to learned hook avoidance of the fish as previously documented for carp (Beukema 1970b; Raat 1985). Because catch rates declined, but carp were observed to continue their foraging activities, learning about hook avoidance seems to have had an impact on catch rates. Learned hook avoidance usually results in reduced numbers of captures, but cues involved in learned hook avoidance behaviour of the fish can be manifold. Similar to the different cues involved in learning about natural predators, antipredator responses at increasing levels of risk (like increasing fishing pressures) include predator detection, recognition and assessment, and finally predator avoidance through flight responses (Kelley & Magurran 2003). Learning about improved responses may arise at any of these stages, but opportunities for learning are dependent on the available visual, olfactory, tactile or auditory cues

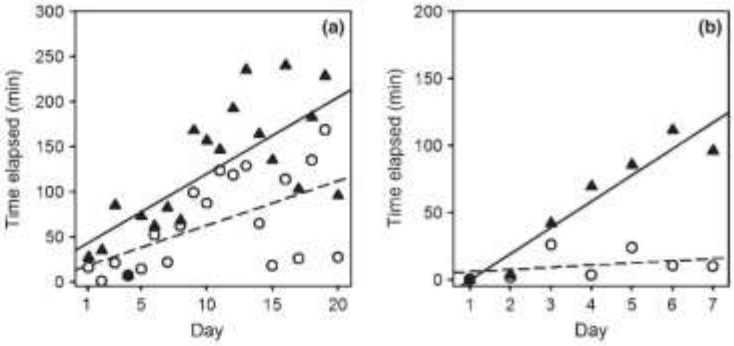

Figure 3. Time elapsed until the first bite of the fishing day (white circles, dashed line) and the time elapsed until the first capture of the day (filled triangle, solid line) in relationship to the number of consecutive angling days within ponds (a) and laboratory tanks (b).

(Kelley & Magurran 2003). Because predator detection, recognition and assessment are dependent on visual cues (Hartman & Abrahams 2000; Walling *et al.* 2004), it is conceivable that carp learned to identify the baited hook visually or learned to expel the angling bait before getting hooked. If fish learned to identify hook and line visually, catch rates should increase under environmental conditions of low visibility. Indeed, within the laboratory, carp were increasingly more vulnerable to angling during darkness with increasing fishing time and similarly, within the consistently turbid ponds, no effects of diel period on angling captures were observed. Further, if tactile cues were important in hook avoidance, fish should have ingested the angling bait, but learned to avoid getting hooked. Within the laboratory, the time until the first bite of the day remained constant over the course of the study and was not related to the time elapsed until the first capture of the day. Thus, carp ingested the angling bait, but avoided getting hooked, supporting the assumption that tactile cues were also involved in learned hook avoidance. No such effects were found within the ponds, also indicating differences in hook avoidance behaviour within different environments.

Another reason for declines in catch rates over time could be that fish reduced the number of visits to the angling locations, thereby reducing their encounter rates with the baited hook. Using a similar experimental setup, Klefoth *et al.* (2012) found that scaled and mirror carp considerably reduced their number of visits at angling locations within the laboratory, once angling started. Thus, reduced catch rates with increasing fishing duration might be related to behavioural responses of the fish towards angling-induced risk. However, within the pond environment, no such effect of angling activities on carp behaviour was observed (Klefoth *et al.* 2012); most likely because permanent latent natural predation risk induced by fish-eating birds and olfactory cues of predatory fish (despite the absence of predatory fish) was present within the pond environment, so that angling activities did not add appreciable risk-stimuli within this environment (Klefoth *et al.* 2012). Based on these findings, reduced catch rates with increasing fishing time can partly be explained by active angling location avoidance of the fish. However, the findings of Klefoth *et al.* (2012) also indicate that behavioural alterations as a response towards angling activities are dependent on the environment, and are less pronounced in more natural pond conditions. In conclusion, hook avoidance seems to be based on different responses towards angling activities including visual and/or tactile identification of the baited hook and potentially reduced visits of previously save feeding spots.

No evidence of differential learning abilities to avoid being hooked between scaled and mirror carp was found, and thus, the hypothesis was not supported. It appeared that differences in adaptation to low-risk aquaculture conditions did not impact the ability to learn to avoid hooking. This finding disagrees with studies comparing adapted learning abilities between fish originating from high- and low-risk environments (Huntingford & Wright 1989, 1992). Reasons for this opposed finding might be related to the experimental setup where groups of fish were tested together in the same ponds and laboratory tanks. Fish are generally able to make decisions about predators based on the behaviour of other fish (Pitcher *et al.* 1986) so that antipredator responses can be optimised through observations of conspecifics that have been treated by a predator (Magurran & Higham 1988; Krause 1993; Mathis *et al.* 1996). Similarly, in the present study carp of both genotypes might have learned to optimise their behaviour towards baited hooks based on observations of conspecifics. This might potentially explain rapid decreases in catch rates, but also observed approximations in vulnerability of scaled and mirror carp over the course of the fishing trials, and non-existing differences in learning abilities between scaled and mirror carp.

In the present study a certain fraction of carp was completely invulnerable to capture (45% and 15% of mirror carp and 68% and 45% of scaled carp within ponds and the laboratory, respectively) and relatively more fish of both genotypes were caught within the laboratory. In comparison, using a comparable experimental approach, Beukema (1969) found 18% of domesticated mirror carp and 33% of less domesticated scaled carp to be invulnerable to angling. These results show that environmental factors can influence the vulnerability of fish (Kuparinen *et al.* 2010), but also confirmed that individuals of the same species can differ in their intrinsic vulnerability to angling gear, as shown previously for carp and other species (Bennett 1954; Beukema 1969; Dunham *et al.* 1986; Raat 1985; Philipp *et al.* 2009). Reasons for the observed higher vulnerability of all carp within the laboratory environment were most probably related to the lack of alternative natural foraging opportunities (Klefoth *et al.* 2012). Thus, carp within the laboratory were forced to trade-off the risk of being captured and active foraging on the angling locations. Trade-off decision making between foraging and risk-taking usually depends on the hunger state of the fish (Cerri & Fraser 1983) where starved and hungry individuals tend to take higher risks than less hungry conspecifics (Godin & Crossman 1994; Damsgård & Dill 1998). The lack of natural food in the laboratory might have forced the fish to visit feeding locations despite being risky, leading to higher catch rates within the laboratory.

Potential explanations for higher vulnerability of mirror carp compared with scaled carp were derived from additional behavioural experiments under laboratory conditions. When observed solitary in aquaria, mirror carp showed higher swimming activities, ingested more food items and took significantly less time to ingest the first food item than their scaled conspecifics. All of these behavioural differences between the two genotypes very likely contributed to observed differences in vulnerability. For example, high activity during foraging likely increases the probability of encountering the baited hook, comparable to higher food encounter rates of more active swimming fish under natural conditions (Boisclair 1992). Similarly, ingesting more food increases the chances that a baited hook is ingested and a mistake is made during the sorting process and testing of ingested baits (a behaviour expressed by the carp after some exposure to angling, see Appendix S1). Increased foraging activity and higher numbers of visits at feeding locations of mirror carp compared with scaled carp have been documented for the same fish used in the present study also in the large laboratory tanks and ponds (Klefoth *et al.* 2012). In the study of Klefoth *et al.* (2012), mirror carp behaved consistently bolder than scaled carp in ponds, independent of angling activities and also mirror carp in the laboratory were found to forage more intensively at the angling locations, once fishing started. Although no differences in the number of visits at the feeding locations were observed in the laboratory before angling started, under the risk of being captured, mirror carp showed consistently higher foraging activities (Klefoth *et al.* 2012). Such behaviour can be interpreted as higher boldness level (i.e. feeding in the presence of predation risk) by the more domesticated mirror carp, which together with greater consumption of food is the most likely explanation for the greater vulnerability to capture by mirror carp relative to scaled carp. Indeed, in carp risk-taking behaviour, competitive ability, and metabolic rate are positively correlated (Huntingford *et al.* 2010). Because risk-taking behaviour, swimming activity and metabolic rate usually increase during the domestication process as a consequence of artificial selection for traits like high growth rates (Price 1999; Martin-Smith & Armstrong 2002; Biro & Post 2008; Huntingford *et al.* 2010), elevated food consumption rate and speed (this study) and generally bolder foraging behaviour (Klefoth *et al.* 2012) might have cumulatively increased the likelihood of catching mirror carp compared with scaled carp.

While boldness behaviour most likely explains higher vulnerability to angling of mirror carp, selective food preferences can be excluded as a reason for the observed patterns. Corn kernels were used as bait during angling experiments, and scaled and mirror carp equally preferred corn kernels over pellets. Carp are known for their selective feeding and taste preferences (e.g. Jönsson 1967; Appelbaum 1980; Kasumyan & Morsy 1996), and domesticated mirror carp are known to prefer artificial pellets over natural food resources (Suzuki *et al.* 1978). However, in the present data the artificial bait corn kernel was preferred by both genotypes. Although no directed attractiveness has been associated with corn kernels when used without additional treatment (Jönsson 1967), common carp quickly locate and forage on artificial and novel food resources like corn kernels in natural environments, likely because it is more easily ingested than natural food (Bajer *et al.* 2010). The observed preferences for corn kernels in the present study could thus be based on taste preferences or other properties of the two types of food like hardness. Although carp are able to ingest, chew and digest hard particles like mussels and snails (Sibbing 1988), or angling baits such as boilies (Niesar *et al.* 2004), preferences for soft food items were reported repeatedly (Jönsson 1967; Stein *et al.* 1975; Sibbing 1988). Thus, preferences for corn kernels over pellets might be best explained by particle hardness, rather than taste preferences. Overall, however, bait preferences could be excluded as an explanation for the differential vulnerability to angling by the two genotypes of carp.

In conclusion, the present study confirmed previous findings of higher angling vulnerability of mirror carp compared to scaled carp, but differed from earlier work in terms of producing common garden reared fish from a common breeding stock. Therefore, the present study provided conclusive evidence for a genetic basis of angling vulnerability in carp, complementing work conducted in other fish species (Dunham *et al.* 1986; Philipp *et al.* 2009). Consequently, it can be predicted that anglers would remove mirror carp-like genotypes (i.e. individuals expressing high level of boldness and food consumption) from a natural population of fish when harvesting occurs at a high rate leaving behind scaled carp-types, which are more tame individuals. Alternatively, the quick learning of carp to avoid future capture introduces a new form of common pool-resource problem in fisheries that targets trophy carp based on a catch-and-release context (Arlinghaus 2007) as there should be a race for first capture among anglers to take advantage of initial high vulnerability. Finally, the present study shows that boldness represents a key phenotypic axis affecting the vulnerability of fish. This contradicts literature reporting more shy individuals to be more readily captured in other species (Wilson *et al.* 2011). Differences among these studies might be related to species-specific patterns or methodological aspects of study designs. Future studies should try to identify the phenotypic correlates of angling vulnerability on the individual level in a variety of species to specify phenotypic traits on which angling-induced selection is acting (Uusi-Heikkilä *et al.* 2008). This work is then the basis to understand the direction of change to be expected from fisheries-induced selection (Jørgensen *et al.* 2007).

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Paper VII

VII

Anna Kuparinen, Thomas Klefoth, Robert Arlinghaus, 2010. Abiotic and fishing-related correlates of angling catch rates in pike (*Esox lucius*). Fisheries Research, 105:111-117.

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Abiotic and fishing-related correlates of angling catch rates in pike (*Esox lucius*)

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ABSTRACT

Understanding how angling catch rates vary between environments is of interest from ecological and fisheries management perspectives, but this has rarely been investigated in detail. Using experimental catch-and-release angling records for northern pike (*Esox lucius*) from a small natural lake in Germany and a generalized linear model we investigated how abiotic and fishing-related environmental variables as well as time of day affect pike catch per unit effort (CPUE; fish per hour). Catch rates of pike were significantly increased at low temperatures, high wind speeds and around full and new moon as well as during dusk. Large fishing effort during the past two days reduced catch rates significantly, indicating the combined influence of abiotic and human-induced variables on the catch rates of pike with angling gear. Of all the significant covariates, fishing effort had the most pronounced effect on catch rates. Our results indicate that anglers can increase catch rates by choosing appropriate weather conditions and lunar phases, but that continuously intensive fishing negatively affects future catch rates even in the absence of harvest. This has implications for the choice of sampling effort using angling gear when attempting to assess fish stocks.

1. Introduction

Angling is a popular recreational and commercial fishing method. While angling methods usually involve rod-and-reel in recreational fisheries, long-lining is the most important angling technique in commercial fisheries. Understanding how angling catch rates vary with environmental variables is of interest from ecological and fisheries management's perspectives. Angling requires fish to attack/ingest the bait or the artificial lure, so that angling catch rates provide insights into the activity and feeding patterns in fish and potentially level of aggressive attacks in predatory fish. In particular, catch rates in angling fisheries should depend on the foraging activity and hunger level of fish as well as their ability to locate or avoid the bait or lure (Uusi-Heikkilä et al., 2008). These processes, in turn, are likely affected by environmental cues correlated with activity and metabolism such as water temperature (Stoner, 2004). Therefore, quantifying the vulnerability of fish to angling gear necessitates identifying environmental variation in angling catch rates, but this has rarely been investigated in detail using rod-and-reel-type angling. Moreover, as stock assessments are sometimes conducted using angling methods (e.g., Myers and Worm, 2003; Pierce and Tomcko, 2003; Hansen et al., 2005; Lehtonen et al., 2009), distinguishing environmental variations in angling catch per unit effort (CPUE; an index of relative abundance) from variation arising from differences in population density is vital for obtaining reliable information about the population size (Stoner, 2004).

Out of the potential abiotic factors affecting angling catch rates water temperature appears to be the variable most commonly reported in the literature (e.g., Bigelow et al., 1999; Stoner, 2004; Stoner et al., 2006; Damalas et al., 2007; Ortega-Garcia et al., 2008). This is presumably due to its pervasive influence on movement activity, metabolism, and foraging activity in all poikilothermic aquatic animals (Brown et al., 2004). Other abiotic environmental variables such as wind speed, light, barometric air pressure, day length, time of day and air temperature have also been shown to affect catch rates in angling fisheries (e.g., Millar et al., 1997; Bigelow et al., 1999; Margenau et al., 2003; Stoner, 2004; Wall et al., 2009). However, particularly in recreational fisheries, analyses of such relationships are sparse, presumably due to the lack of datasets providing high resolution measurements of abiotic environmental variables along catch records (Stoner, 2004). This lack of knowledge contrasts with a wealth of anecdotal information about correlations between abiotic environmental variables and angling catch rates, culminating in fishing 'calendars' that are commonly applied by anglers to predict future fishing success.

A particularly intriguing aspect related to abiotic environmental variations in catch per unit effort (CPUE) is the potential role of lunar cycles. Namely, it is a common belief among professional and recreational anglers that catch rates depend on the moon

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phase, with catches being highest around full and new moon (e.g., http://www.solunarforecast.com/). In the context of baited fishing gears, this hypothesis has rarely been investigated and the available evidence is mixed. In some longline fisheries for marine species, catches have been seen to peak around full or new moon (Bigelow et al., 1999; Lowry et al., 2007; Damalas et al., 2007), while other studies have reported lack of correlations between lunar phases and catch rates (Millar et al., 1997; Ortega-Garcia et al., 2008). Overall, the extent to which lunar cycles might affect CPUE in angling is still largely unknown and may be species-specific. Moreover, as pointed out by deBruyn and Meeuwig (2001), weak lunar cycles in catch rates easily remain undetected if the statistical methods that are applied are not appropriate.

In addition to abiotic environmental variables, it is undisputed that a number of biotic features affect catch rates of angling gears. For example, food abundance and the density of conspecifics generally increase competition for food or induce social stress in cannibalistic species (Edeline et al., in press), which may affect food intake rates, foraging activity and hunger levels. Not surprisingly, density-dependent factors have been reported to substantially affect catchability in angling fisheries (Raat, 1986, 1991; Hansen et al., 2005). In some species, angling catchability also depends on learning to avoid capture, particularly if catch-and-release fishing is widespread (Raat, 1985; van Poorten and Post, 2005; Askey et al., 2006), but the same pattern can also emerge as a result of high angling effort with easily-identifiable lures as was demonstrated by Beukema (1970). In his catch-and-release experiment conducted in ponds, northern pike (*Esox lucius*) learned to avoid future capture by artificial lures regardless of whether individuals were hooked previously, but similar learning effect did not occur for natural baits (Beukema, 1970). This finding along with other studies in freshwater fisheries conducted with artificial lures (van Poorten and Post, 2005; Askey et al., 2006) suggests that fishing effort might affect angling catch rates negatively (Cox and Walters, 2002; Young and Hayes, 2004). Thus, fishing effort must be accounted for when investigating the impact of environmental factors on catch rates in angling fisheries.

The objective of this study was to investigate the impacts of a wide range of abiotic and fishing-related environmental variables including lunar cycles and fishing effort on CPUE in northern pike (hereafter termed pike) rod-and-reel angling. Pike is a fast growing, early maturing and strongly cannibalistic top piscivore in freshwater and brackish ecosystems; it was chosen as the model species for the present study because it has great value for both commercial and recreational fisheries throughout its circumpolar natural range in the northern hemisphere (Paukert et al., 2001; Arlinghaus and Mehner, 2004). Field data were collected by experimental catch-and-release fishing in a natural lake. The study site was protected from any other forms of fishing and was confined to a short period of intensive sampling within one season. Therefore, variations in CPUE were likely not associated with large changes in population density, which would otherwise confine the analyses of abiotic and fishing-related variations in catch rates (Hansen et al., 2005; VanDeValk et al., 2005).

2. Materials and methods

2.1. Data

Experimental pike angling took place in the Kleiner Döllnsee during the spring to autumn of 2005. This small (25 ha), dimictic, shallow (mean depth 4.1 m, maximum depth 7.8 m) natural lake is located in north-east Germany (N52°59′, E13°34′). Kleiner Döllnsee is mesotrophic to slightly eutrophic (P concentration at string overturn 28 $\mu g\, l^{-1}$) sustaining a natural pike population protected from any form of commercial or public recreational fishing (Klefoth et al., 2008; Kobler et al., 2008a). In spring of 2005, abundance of age 1 year and older pike was estimated as 544 individuals (95% CI: 194–1088) (Kobler et al., 2008a,b). Within the study period from 27th May to 17th September 2005 pike were angled on a total catch-and-release basis using 25 skilled anglers (3 fishing regularly on each sampling day, occasionally supplemented by in total 22 additional experimental anglers). Most the anglers were part of the research team and all employed standard recreational pike angling fishing techniques described in detail in Arlinghaus et al. (2008a). Briefly, each angler was instructed to use a personal choice of artificial lures using medium-action rods for spinning or trolling and, when occasionally using organic bait, to set the hook quickly to avoid deep hooking (Arlinghaus et al., 2008a). Anglers were asked to fish all habitats during a fishing day, but for logistical reasons and to add realism anglers were not assigned to randomly selected fishing sites. Due to the small size of the lake all available habitats were sampled on a given angling day, but some more productive fishing sites (e.g. dense macrophyte patches) known by anglers to host particularly high abundances of pike (see Kobler et al., 2008a,b, 2009) might have been more intensively fished on particular days. However, this is typical for any recreational fishing sites and, hence, managers would normally have aggregated daily catches over a sample of anglers. Once a pike was landed, it was quickly de-hooked, checked for any signs of marks or tags, and its length and weight were measured after which the individual was released. Immediate hooking mortality was low and estimated as 3.9% (see Arlinghaus et al., 2008a for details). For each angling day, the cumulative number of pike caught and the cumulative duration of fishing over all anglers was recorded, separately for daytime and the hours of dusk, yielding 169 observations in total (93 during daytime and 76 during dusk spread over 94 fishing days; Fig. 1). For more ecological details about the study system, see Klefoth et al. (2008) and Kobler et al. (2008a,b, 2009).

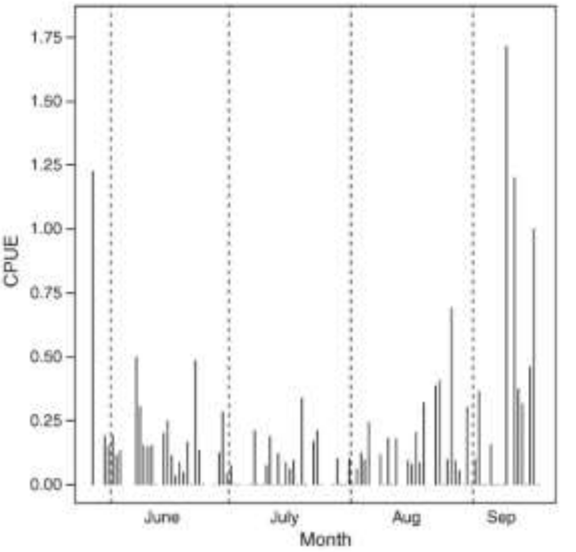

Fig. 1. Pike catch per unit effort (CPUE; fish per hour) at daily intervals over the study period from 27th May to 17th September 2005.

Abiotic environmental conditions were measured on a daily basis. Variables measured (and their ranges) were water temperature (14.7–24.1 °C), wind speed (0.7–6.9 $m\, s^{-1}$), wind direction [categories (frequencies of observation): east (14), south (38), west (40), north (2)], air humidity (58–98%), rain

Table 1
Pearson's correlation coefficients (r) among the continuous environmental variables.

	Air pressure	Future air pressure	Humidity	Hours of sunshine	Rain	Water temperature	Wind speed
Air pressure	1	−0.319	−0.393	0.450	−0.314	−0.125	−0.312
Future air pressure		1	0.373	−0.320	0.149	−0.074	0.200
Humidity			1	−0.762	0.480	−0.267	0.139
Hours of sunshine				1	−0.412	0.318	−0.346
Rain					1	−0.062	0.094
Water temperature						1	−0.206
Wind speed							1

Table 2
Effects of the significant covariates on the number of pike caught per hour, as estimated through a generalized linear model with a log link and Poisson errors[a] ($N = 169$).

Model term	Coefficient (SE)	Deviance[b]	p-Value
Intercept (time of the day: daytime)	0.477 (0.828)		
Past two day fishing effort	−0.019 (0.005)	17.83 (df = 1)	<0.001
Time of the day: dusk	0.556 (0.181)	8.58 (df = 1)	0.003
Water temperature	−0.095 (0.037)	6.40 (df = 1)	0.011
Wind speed	0.160 (0.061)	6.66 (df = 1)	0.010
$\cos(2\theta)$	0.238 (0.114)	4.52 (df = 1)	0.034

[a] Null deviance 209.01 (df = 169), residual deviance 168.82 (df = 163).
[b] Marginal increase in residual deviance upon deletion of the term.

(0.0–14.3 l m^{-2}), hours of sunshine (0–15 h day^{-1}), and air pressure (997.8–1024.6 hPa) and change in air pressure within the following 24 h (−11.4–12.8 hPa). Generally, the measured variables did not show strong correlations (Table 1), except humidity and the hours of sunshine for which Pearson's correlation coefficient was <−0.5. The daily moon phase at the geographic location of the study site was obtained from the Naval Oceanography Portal (http://aa.usno.navy.mil/data/docs/MoonFraction.php), and was expressed in terms of the fraction of moon disk illuminated and whether the moon was waxing or waning. As an index of angling pressure on the ecosystem, fishing effort (hours of angling) was calculated jointly for the first two days prior to the sampling day (i.e., total hours of fishing over the two days), and separately for the third and fourth day prior to the sampling day (hours of fishing over the respective day).

2.2. *Statistical analyses*

Our aim was to investigate whether daily abiotic and fishing-related environmental variables affected pike catch rates with typical recreational angling gear during day and dusk. To this end, the number of pike caught (daily catch split into two categories for the time of the day) was modelled with a generalized linear model (GLM) with a log link function and Poisson errors, and corresponding angling hours as an offset variable. Being constructed in this way, the model predicts the pike catch rate in fish per hour. As explanatory variables we considered the recorded environmental variables (see above) and time of the day (daytime or dusk) as a categorical variable. Fishing effort during the previous two days, and during the third and the fourth day prior to the sampling day were used as explanatory variables to account for the known behavioural responses of pike to angling activity-induced habitat disturbance (Klefoth et al., unpublished data), which may translate into reduced catch rates. Lunar cycles in the pike catch were investigated by converting the fraction of moon disk illuminated into radians (θ), so that one lunar cycle corresponded to a gradual increase from 0 to 2π radians (e.g., 0 and 2π radians corresponded with full moon and π radians with new moon). Transformations $\cos(\theta)$, $\sin(\theta)$, $\cos(2\theta)$, and $\sin(2\theta)$ were then included in the model as explanatory variables to investigate possible lunar effects around full/new moon (cosine), around half moon (sine) and for lunar effects peaking twice within one lunar cycle (cosine and sine transformations of 2θ). For more details of this method, see deBruyn and Meeuwig (2001).

Because of the large number of investigated covariate candidates in relation to the number of observations, interaction terms could not be readily included to the model. Analyses were therefore carried out in two consecutive steps: First we fitted a model with the additive main effects of all the covariate candidates. After having identified the significant covariates, we then fitted a model with both the main effects and two-way interactions of the significant environmental covariates. Significance of the covariate candidates was investigated by stepwise reduction of the full model and Chi-squared test of deviance. Possible non-linearity in the effects of the significant covariates was investigated with a generalized additive model (GAM). The impact of potential outliers was investigated by excluding those from the data and repeating the analyses.

Because environmental variables were measured on a daily basis, a potential day effect was not included in the model as it could easily sweep variation in the catch rate assigned to variations in the other explanatory variables. However, to investigate possible daily variations that were not encompassed by the considered explanatory variables and to detect seasonal trends in pike catch rates (Margenau et al., 2003), residuals of the fitted model were further analysed. Relative magnitudes of within and between day variations in catch rates were estimated by modelling the residuals with a linear mixed effect model with a fixed intercept and date as a random effect. A possible temporal trend in catch rates over the study period was investigated by fitting a GAM model to the residuals in which the potential day effect was described though a non-parametric smoothing term. All the statistical analyses were performed in R 2.10.0 (R Development Core Team, 2009).

3. Results

Catch rates of pike fluctuated widely during the study period with no obvious pattern visible from the time series (Fig. 1). The catch rate of pike by angling gear was found to be significantly affected by the past two days' fishing effort, time of the day, and average daily water temperature, wind speed and moon phase (Table 2). Catch rates were significantly increased during dusk, at high wind speeds and around full and new moon, and decreased significantly with increasing water temperatures and when large amounts of fishing took place during the previous two days (Fig. 2). The effects of other explanatory variables were non-significant (in the order of deletion from the model, hours of sunshine: $\chi^2 < 0.0001$, df = 1, $p = 0.998$; fishing

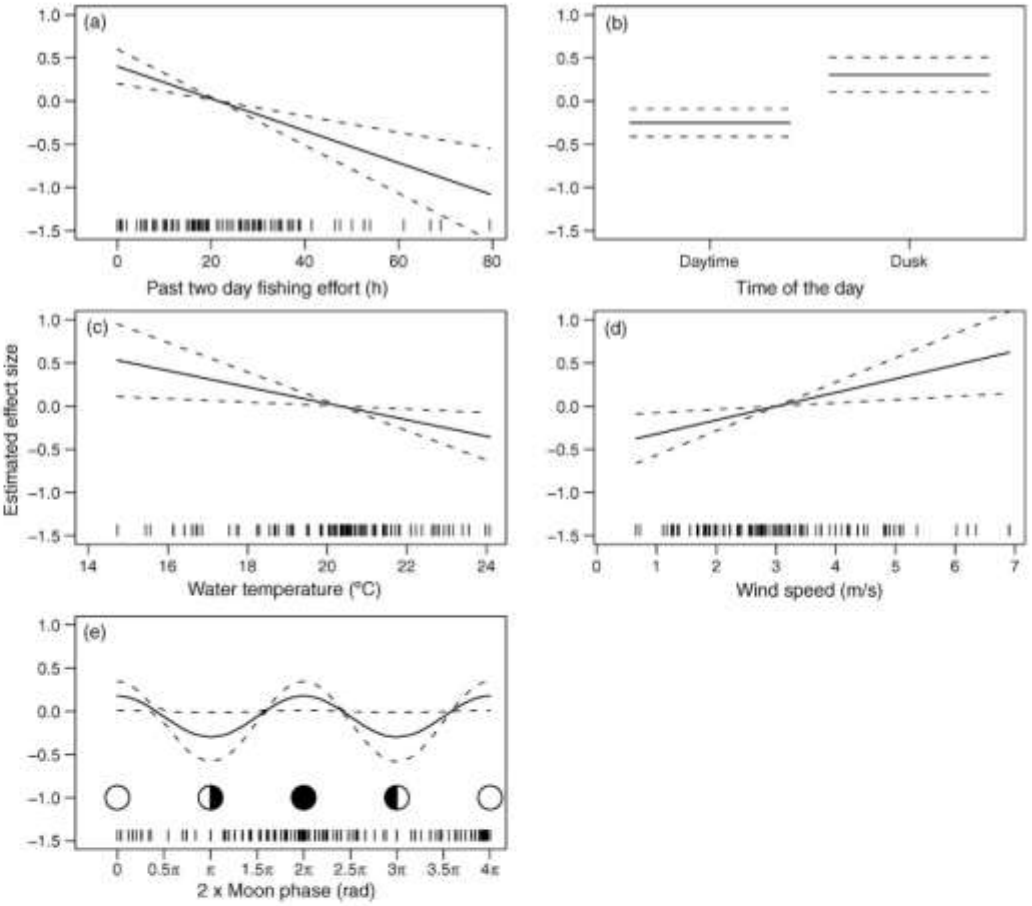

Fig. 2. Partial effects (solid line) of past two day fishing effort (a), time of the day (b), water temperature (c), wind speed (d), and cosine of double the moon phase (e) on the log transformed pike catch rate (fish per hour) predicted by the generalized linear model in Table 2. Standard error ranges are indicated with dashed lines and densities of observations are illustrated with rugs. In case of time of the day categories, there were 93 observations during daytime and 76 during dusk.

effort during the fourth day prior the sampling day: $\chi^2 = 0.0004$, df = 1, $p = 0.985$; air pressure: $\chi^2 = 0.008$, df = 1, $p = 0.960$; wind direction: $\chi^2 = 2.902$, df = 3, $p = 0.407$; fishing effort during the third day prior the sampling day: $\chi^2 = 0.490$, df = 1, $p = 0.484$; $\sin(\theta)$: $\chi^2 = 0.625$, df = 1, $p = 0.429$; air pressure change: $\chi^2 = 1.4$, df = 1, $p = 0.237$; rain amount: $\chi^2 = 1.610$, df = 1, $p = 0.205$; humidity: $\chi^2 = 1.914$, df = 1, $p = 0.167$; $\sin(2\theta)$: $\chi^2 = 2.006$, df = 1, $p = 0.157$; $\cos(\theta)$: $\chi^2 = 3.720$, df = 1, $p = 0.054$). The overall explanatory power of the significant covariates on pike catch rates remained modest, with the reduced model explaining 19.2% of the null deviance and its Nagelkerke's R^2 (Nagelkerke, 1991) being 21.4%. Generally, the model fit was acceptable and there was no evidence of overdispersion, as indicated by the goodness-of-fit test based on deviance ($\chi^2 = 168.82$, df = 163, $p = 0.361$). No interactions were found between the significant covariates on pike catch rates (in the order of deletion, water temperature × $\cos(2\theta)$: $\chi^2 = 0.345$, df = 1, $p = 0.557$; wind speed × $\cos(2\theta)$: $\chi^2 = 0.880$, df = 1, $p = 0.348$; wind speed × time of the day: $\chi^2 = 0.813$, df = 1, 0.367; water temperature × time of the day: $\chi^2 = 0.828$, df = 1, $p = 0.363$; time of the day × $\cos(2\theta)$: $\chi^2 = 1.814$, df = 1, $p = 0.178$; water temperature × wind speed: $\chi^2 = 2.346$, df = 1, $p = 0.126$). GAM analyses did not reveal deviations from linearity in the effects of fishing effort, water temperature, and wind speed on catch rate (judged visually). Residuals, leverages and Cook's distances pointed out four possible outliers but excluding those from the data had no effect on the outcome of the analyses. Variation in residuals could not be encompassed by daily random effects (the variance component was 6.8×10^{-7} and thus virtually zero), and no significant temporal patterns in residuals were detected by a smoothed day effect ($F_{2.541,169} = 1.867$, $p = 0.147$).

4. Discussion

Our results illustrate how angling catch rates of a common predatory freshwater fish vary within and between days due to variation in abiotic and fishing-related environmental variables. From a wide range of potential covariates pike catch rates were found significantly correlated with averages daily water temperature, wind speed, and moon phase, as well as with the timing of fishing within the day and the previous two day fishing intensity levels (Fig. 2), together inducing one order of magnitude variation in the predicted catch rates (0.05–0.44 fish per hour). However, despite the distinguished role of these covariates, a large amount of variation in the observed catch rates remained unexplained (Table 2). This suggests that a good deal of stochasticity remained associated with fishing success. This could have arisen from differences in bait or lure types employed by experimental anglers across sampling days (Arlinghaus et al., 2008a; Alós et al., 2009), angler's skills (Alós et al., 2009) or varying spatial distribution of fishing across sites in the study lake (Alós et al., 2009). These variables could not be accounted for in our analyses, but they are not expected to bias the results of our analyses for two reasons. First, variation arising from anglers and sites should not be large as data were averaged over these variables and the negligible variance component of the day effect suggests that no variation was associated with daily combinations of anglers. Second, anglers and sampling of sites within the lake were generally independent of the considered environmental covariates, so that they would only add to the residual variation of the model but not affect our inference on significant environmental covariates of the pike catch rate. Additional variation in catches might also have arisen from within-day variations in environmental conditions (e.g., temperature or oxygen fluctuations), from variation in the size of pike population vulnerable to angling in the course of the study resulting from recruitment or natural mortality, from unaccounted environmental drivers such as prey fish distribution, or from deviations from model assumptions. Nevertheless, the presence of unexplained variation in catch rates does not affect the robustness of our findings, which show that abiotic environmental variables as well as fishing effort significantly affected catch rates in pike angling. Moreover, it should be noted that all the mentioned sources of uncertainty are typically present in recreational angling catch data, thus increasing the realism of our angling experiment from the perspective of practical fisheries management. In other words, the environmental patterns seen in our catch rates are likely to be present in pike angling catch rate records typically available unless there are substantial differences in environmental covariates of pike catch rates across lakes or rivers. This is currently unknown and warrants future research and replication.

The previous two days of fishing effort at the study lake turned out to be a much more important predictor of the pike catch rate than any meteorological or lake-specific abiotic variable (Table 2). This result is in line with previous observations that fish, including pike (Beukema, 1970; Klefoth et al., unpublished data), temporarily alter their behaviour in response to human-induced disturbances such as catch-and-release events, habitat disturbance through boating, or presence of anglers and associated sound originating from the fishing activity (e.g., Raat, 1985; Young and Hayes, 2004; Askey et al., 2006). However, this effect has been found to be rather short term in pike, lasting a couple of days (Klefoth et al., 2008) or just some hours (Arlinghaus et al., 2008b, 2009). In our study, a potential 'memory' of pike to avoid future capture appeared to extend only up to two days back as reflected by the non-significant effects of the daily fishing effort three and four days prior to the sampling day. This is also indicated by the observation that after the start of intensive angling on this otherwise unexploited pike population there was no sudden and consistent drop in catch rates as has been shown for other freshwater fish species (Raat, 1985; van Poorten and Post, 2005; Askey et al., 2006) suggesting comparatively low levels of learning to avoid future capture by pike. However, extrapolations to other systems should be done cautiously because fishing effort more intensive than that in our study might have longer term effects (compare Beukema, 1970). Moreover, in fish habitat size and complexity can affect sensitivity to remembering negative stimuli (Pollock and Chivers, 2003) as well as the development of brain and sense organs (Pollen et al., 2007), and this might explain the contrasting findings by Beukema (1970) from pond-angled pike that showed rapid learning to avoid capture by lures, but not by natural bait, in this low complexity environment. Therefore, we speculate that the negative impact of fishing effort on angling catch rates might be less pronounced in larger and more complex ecosystems than the one we sampled. An alternative reason for our findings unrelated to a short-term memory hypothesis might be that in a given point in time pike populations might cluster into individuals temporarily vulnerable and invulnerable to fishing, e.g. due to short-term elevated hunger levels or habitat choice (Cox and Walters, 2002). If this is the case heavy fishing pressure in a short time period of two days might temporarily remove a great fraction of the vulnerable pool leaving behind more invulnerable fish, which, in turn, reduces catch rates. Irrespective of the exact mechanism responsible for declining catch rates with short-term elevated fishing effort, several practical implications can emerge from our observations. First, to ensure high catch rates it might be worthwhile to distribute fishing effort equally in time and allow the population to 'recover' between pulses of intensive fishing (cf. van Poorten and Post, 2005). Secondly, if catch rates in angling are applied as a proxy for population density (e.g., Pierce and Tomcko, 2003; Lehtonen et al., 2009), fishing effort over the previous days should be kept constant because it influences catch rates and might therefore influence the abundance index derived using angling gear.

We found that increasing water temperature from about 14 °C to the maximum observed temperature of about 24 °C was associated with decreasing pike catch rate. This temperature range coincides well with earlier studies on catch rates of pike with stationary gill nets in lakes: outside spawning time catch rates were found to be highest at temperatures between 15 and 17 °C and lowest at the maximum temperatures between 20 and 24 °C (Casselman, 1978). Although active swimming may not necessarily be expected for successful foraging for a predator such as pike regularly engaging in a sit-and-wait hunting strategy, direct correlations exist between the rate of food consumption, swimming activity and water temperature in this species (Casselman, 1978). Casselman (1978) also reported that for adult pike rapid somatic growth commences at about 14 °C after spawning in spring because appetite to restore lost energy resources is stimulated and pike actively seek food. With further increasing temperature, instantaneous growth and activity of juvenile pike have been shown to increase in laboratory experiments but after reaching a threshold value of about 19–20 °C both growth and activity of pike decline (Casselman, 1978). Because in pike the optimum temperature for growth and activity decrease with age, and food supply is often limited under natural conditions, Casselman (1978) concluded that maximum activity of pike in natural environments should occur at low temperature ranges <20 °C. This would match with the inverse relation between temperature and pike catch rates by angling found in the present study. Presumably, in this mesothermal or 'coolwater' fish species (Casselman, 1978) overly high water temperature during summer may pose physiological stress, particularly if food supply is limited, because for metabolic reasons it would be more efficient (Bevelhimer et al., 1985) if swimming activity and feeding is reduced (Casselman, 1978). However, also an alternative reason might help to explain the inverse relation between water temperature and pike catch rates found in the present study. Elevated temperature during the warming spring and summer period coincides with high movement activity (Jacobsen et al., 2004) and abundance of prey fish such as roach (*Rutilus rutilus*) (Kobler et al., 2009). Because the encounter probability with naturally more active prey is increased at high water temperature, the angler's lure and bait might face a 'competitive disadvantage' reducing catch rates. Along the same lines, we can suspect that the higher catch rates of pike at colder water temperatures (see also Margenau et al., 2003) could be associated with parallel declines in prey movement activity (Jacobsen et al., 2004) coupled with the tendency of pike to keep being associated with underwater structure as refuge and shelter even at low water temperature (Kobler et al., 2008a). This facilitates the identification of pike habitat by anglers (Post et al., 2002), which at lower temperature coincides with reduced prey encounters and presumably higher hunger levels of pike, jointly increasing the pike catch rates by angling.

In our study, we also found pike to be more vulnerable to angling during twilight periods. During these periods pike are usually more active in terms of swimming (Kobler et al., 2008a,b) and feeding (Casselman, 1978) because prey fish start to emerge from shelter and disperse in the open water (Jacobsen et al., 2004) and because pike are able to approach their prey closer and therefore the chances of successful attacks are increased (Pitcher and Turner, 1986). However, greater fishing success at twilight conditions might also be explained because during the dusk period a visual predator such as a pike might have greater difficulties in identifying (and consequently avoiding) an artificial lure than during day time. Similarly, we suspect that the positive effect of high wind speeds on catch rates of pike might be associated with the fact that strong winds tends to induce turbidity (e.g., Cózar et al., 2005), which is known to affect foraging and feeding activity of pike (Nilsson et al., 2009) and distribution of prey like roach (Jacobsen et al., 2004) and small perch (*Perca fluviatilis*) (Skov et al., 2007). Thus, increased wind speeds can alter the reactivity of pike to the fishing gear by reducing water transparency (e.g., Stoner, 2004) and/or altering prey encounters, thus jointly affecting the susceptibility of individual pike to lures. This speculation of the catch rates being enhanced by reduced water transparency as a result of wind speed is in agreement with the observation made by Casselman (1978) that pike feed more actively on cloudy, overcast days than on bright, sunny days.

One of the most intriguing and novel finding of our study is the clear relationship between moon phase and catch rates in pike angling. Interestingly, the shape of the moon effect detected in pike catch rates with catch rates peaking around full and new moon matches well with that predicted by anecdotally supported fishing calendars. Our study therefore adds scientific weight to the usefulness of fishing calendars that are based on moon phases. What our analysis does not reveal, however, are the mechanisms underlying the observed pattern. Typical secondary moon effects arising from tidal formations or illumination (Kuparinen et al., 2009) may be thought of as not playing an important role in pike as the lake environment was free from tides and fishing took place during daytime and dusk, so that the potential effect of moonlight can be excluded. However, it is possible that shifts in illumination along lunar cycles are associated to periodic changes in zooplankton and prey fish distribution, leading to changes in predator foraging activities that transcend the night phases (Hernández-León, 2008). Thus, it might also be conceivable that predators such as pike respond to signals of the moon that correlate in a predictable and repeatable way with altered distributions of both zooplankton and prey fish. Direct lunar gravitational patterns might offer one possible explanation as a biological trigger of a behavioural response by predators because those have previously been found to induce behavioural reactions in fish, e.g. inducing migration of smolts in salmonid species (DeVries et al., 2004). However, mechanisms through which pike might sense gravitational cues, and the biological role these might have in motivating feeding and, thus, vulnerability to angling remains unknown providing a challenge for future research.

To conclude, our study sheds light onto some important correlates of catch rates in angling by rod-and-reel for pike. Although a substantial amount of variation in catch rates remained unexplained, we found daily averages of abiotic variables (water temperature, wind speed, lunar phase), time of the day, and fishing variables (angling effort) to significantly affect catch rates. Our study is useful in directing future attempts to assess the size and structure of pike stocks based on angling catches by emphasizing the importance to account for environmental variables significantly influencing catch rates. This can be most easily achieved by randomly selecting sampling days and avoiding temporal clustering, so that a large environmental gradient is sampled. Moreover, our study confirmed some of the anecdotal evidence by anglers about the seasonality and diurnal dynamics of pike catch rates that seem to be also mediated by lunar cycles. This information is important for anglers interested in maximizing their catches by allocating scarce fishing time to the most productive periods within a season and throughout the day.

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Paper VIII

VIII

David A.H. Sutter, Cory D. Suski, David P. Philipp, Thomas Klefoth, David H. Wahl, Petra Kersten, Steven J. Cooke, Robert Arlinghaus, 2012. Recreational fishing selectively captures individuals with the highest fitness potential. Proceedings of the National Academy of Sciences of the United States of America, 109:20960-20965.

Recreational fishing selectively captures individuals with the highest fitness potential

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Fisheries-induced evolution and its impact on the productivity of exploited fish stocks remains a highly contested research topic in applied fish evolution and fisheries science. Although many quantitative models assume that larger, more fecund fish are preferentially removed by fishing, there is no empirical evidence describing the relationship between vulnerability to capture and individual reproductive fitness in the wild. Using males from two lines of largemouth bass (*Micropterus salmoides*) selectively bred over three generations for either high (HV) or low (LV) vulnerability to angling as a model system, we show that the trait "vulnerability to angling" positively correlates with aggression, intensity of parental care, and reproductive fitness. The difference in reproductive fitness between HV and LV fish was particularly evident among larger males, which are also the preferred mating partners of females. Our study constitutes experimental evidence that recreational angling selectively captures individuals with the highest potential for reproductive fitness. Our study further suggests that selective removal of the fittest individuals likely occurs in many fisheries that target species engaged in parental care. As a result, depending on the ecological context, angling-induced selection may have negative consequences for recruitment within wild populations of largemouth bass and possibly other exploited species in which behavioral patterns that determine fitness, such as aggression or parental care, also affect their vulnerability to fishing gear.

recreational fisheries | reproduction | evolutionary change | catchability | angler

Size-selective fishing, or even just an elevated level of fishing mortality, has the potential to induce rapid evolutionary change in a range of production-related traits in fish populations (1, 2). Theoretically predicted and empirically supported fisheries-induced adaptive change involves the modification of life history traits, including reductions in age- and size-at-maturation, increases in reproductive investment, and changes in pre- and/or postmaturation growth rates (1–3). Changes in life history traits in response to fishing often collectively reduce adult size-at-age and fisheries yield and result in fish populations that only slowly rebound from overexploited states (4–7). There is little consensus, however, concerning the prevalence of fisheries-induced evolution and its relevance to management (1, 8–10). Perspectives range from calls for "evolutionarily enlightened management" (11) to positions that argue that evolutionary change induced by fishing is slow, thereby rendering it largely unimportant to fisheries management (9).

One important tool to predict long-term population-level consequences of fisheries-induced evolution involves the construction and analysis of individual-based models (5, 12) or more simplified stage or age/size-structured (7) population models. Suitable models to study the potential for fisheries-induced evolution include ecological feedbacks resulting in density- and frequency-dependent selection that shapes fitness landscapes and evolutionary responses to fishing (7, 10). Many of these simulation models assume that fitness or fitness surrogates scale positively with body size (e.g., an exponential increase in individual female fecundity with size or a decline in natural mortality with increasing body length) (5, 7, 12, 13). Under such model assumptions, size-dependent fishing mortality often means that larger, more-fit individuals are harvested at a higher rate than smaller, less-fit ones. Several empirical studies on wild fish populations, however, have questioned the notion that larger fish generally exhibit higher reproductive fitness (14, 15). As a result, although for many fisheries size-selective exploitation is well established (3–5, 7), there is little empirical evidence from wild populations demonstrating that fishing truly targets reproductively more-fit individuals. One can speculate that certain fishing gear might even target less-fit individuals, that is, those that are either competitively inferior or in poorer condition and therefore more prone to attack fishing lures, more likely to encounter passive fishing gear, or less able to evade actively fished gear such as trawls.

Although size-selective mortality should be common across many fisheries, the relationship between size and capture probability is gear-dependent, and the resulting size-selectivity curves are often nonlinear (16). Moreover, a trait that renders individuals more vulnerable to capture likely also varies across different taxa and/or fisheries. In some fisheries, such as recreational angling, selection seems to operate on angling vulnerability as a complex, heritable trait composed of a range of covarying physiological, behavioral, and life history traits (17, 18), rather than body size per se. In the popular recreational fish species largemouth bass (*Micropterus salmoides*), for example, it has been shown that fish with higher vulnerability to angling also exhibit higher metabolic rates and more intensive parental care (19–21). Likely as a result of greater energetic expenditure, however, the largemouth bass that were genetically predisposed to being vulnerable to capture did not grow better under experimental pond conditions in which food was limited (20, 21). Because more-vulnerable individuals may have particular behavioral phenotypes during mate choice that are desired by females (e.g., higher levels of aggression that presumably indicate a willingness to provide more intense parental care), they may still be reproductively more fit than their less-vulnerable conspecifics, despite their smaller body size. We addressed this hypothesis in the present study.

The largemouth bass is one the most targeted species in North American recreational fisheries (22), making it an ideal study species to examine the consequences to fitness of recreational angling. Nesting male largemouth bass provide extended parental

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care for several weeks, defending eggs and fry against potential egg predators, which makes them particularly vulnerable to angling during the reproductive period (23, 24). This is important because nest defense behavior of males during parental care has been found to be critical for brood survival (24, 25). In addition, although it has been proposed that fisheries-induced selection in recreational angling operates more directly on behavioral and underlying physiological traits than on body size (17), it is most likely that both body size and the type and degree of male parental care behavior are involved in determining the fitness of individual males (13). That is, larger males are expected to compete better for nesting sites, and as a result of male size-based female mate choice, often have higher mating success (26). It is also likely that the most aggressive males provide the most intensive parental care and as a result are the most vulnerable to fishing during the nesting period (23). This would, independent of individual size, result in the potentially most reproductively fit individuals facing the greatest likelihood of capture and subsequent death or in the case of catch-and-release fisheries loss of fitness due to loss of all or some of their brood to egg predators (23–25).

The objective of the present study was to assess the relationship between reproductive fitness and vulnerability to angling, using as a model system male largemouth bass from two experimental lines selected over three generations for differences in their vulnerability to angling (18). Because angling should selectively target those largemouth bass males that are the most aggressive nest defenders and therefore the more likely to attack angling lures [i.e., males from the line of highly vulnerable (HV) fish], we postulated that under identical ecological conditions these HV males should demonstrate higher levels of reproductive success than the less vulnerable (LV) males. We further postulated that females might also differentially allocate more reproductive resources to HV males because they might recognize and preferentially mate with a superior nest defender, as has been observed in a range of other species that provide male parental care (27).

Results and Discussion

Spawning events occurred in each of six experimental ponds over 30 d (April 21 to May 20, 2009), with 45 of the 48 males (94%) receiving eggs during this period. Even though the spawning period lasted more than 4 wk, the majority of males (56%) spawned within the first week. There were no differences in the relative timing of spawning between HV and LV males, but in each pond the larger males of both lines spawned earlier than the smaller males (Table 1), a pattern seen before for largemouth bass (28). In addition, there was a clear effect of male size and selection line on male mating success (i.e., the number of eggs received by a male in his nest during spawning) (Fig. 1*A* and Table 1), with a strong interactive effect between male size and line (Table 1). That is, larger HV males had substantially greater mating success than smaller HV males, as well as both larger and smaller LV males, indicating that females were allocating more eggs to larger HV males.

Even though there was no observed effect of male size on the intensity of the parental care behavior expressed before egg hatching, there were some significant behavioral differences between HV and LV males (Fig. 2*A* and Table 1). HV males spent a greater proportion of time guarding their nests and fanning their eggs, whereas LV males spent a greater proportion of time away from their nests. The resulting parental care behavioral scores were on average 36% lower for LV than for HV males (Fig. 2*A* and Table 1). During this period HV males also showed significantly higher aggression toward potential brood predators (i.e., they were more than twice as likely to hit hookless fishing lures than were LV males) (Fig. 2*B* and Table 1). These findings

Table 1. Parameter estimates of generalized linear mixed models assessing the relationships of selected line, their respective size, and the date of spawning on reproductive, behavioral, and offspring characteristics

Response variable	Variable	Parameter estimate	*SE*	*df*	*t*	*P*
Date of spawning	Intercept	5.1	0.76	5	6.65	0.001
	HV vs. LV	0.1	0.19	5	0.72	0.505
	Male size	−0.01	0.003	30	−4.26	0.0002
No. of eggs received	Intercept	1,561.2	5,151.83	5	0.30	0.774
	HV vs. LV	13,855.9	6,731.72	5	−2.06	0.095
	Male size	16.4	18.09	22	0.90	0.376
	Male size × HV vs. LV	56.6	23.85	22	2.37	0.027
Diligence of parental care	Intercept	3.6	12.32	5	0.29	0.780
	HV vs. LV	11.1	3.32	5	3.36	0.020
	Male size	0.04	0.04	25	0.96	0.348
Anti-predator aggression	Intercept	−0.9	0.83	5	−1.08	0.331
	HV vs. LV	1.1	0.43	5	2.60	0.048
	Male size	0.003	0.003	27	1.13	0.268
Duration of parental care of swim-up fry	Intercept	11.4	8.96	5	1.27	0.260
	HV vs. LV	−24.5	11.55	5	−2.12	0.087
	Male size	−0.01	0.03	19	−0.25	0.802
	Male size × HV vs. LV	0.1	0.04	19	2.25	0.037
No. of offspring in fall	Intercept	0.6	1.89	5	0.32	0.759
	HV vs. LV	−5.3	2.51	5	−2.10	0.090
	Male size	0.01	0.007	33	1.26	0.216
	Male size × HV vs. LV	0.02	0.008	33	2.27	0.030
Size of offspring in fall	Intercept	56.4	3.24	5	17.43	<0.0001
	HV vs. LV	−2.9	0.86	5	−3.33	0.021
	Date of spawning	−0.3	0.17	18	−1.76	0.095

Largemouth bass males selected for high vulnerability to angling (HV) versus males selected for low vulnerability to angling (LV). The LV line is the reference group (= 0) in all models. The units of the response and predictor variables are: time variables in days (day of the first spawning = 1), size variables in mm, and two ordinal scores (diligence of parental care, range 0 to 36; aggression measured as hits to fishing lures ranging from 0 to 15).

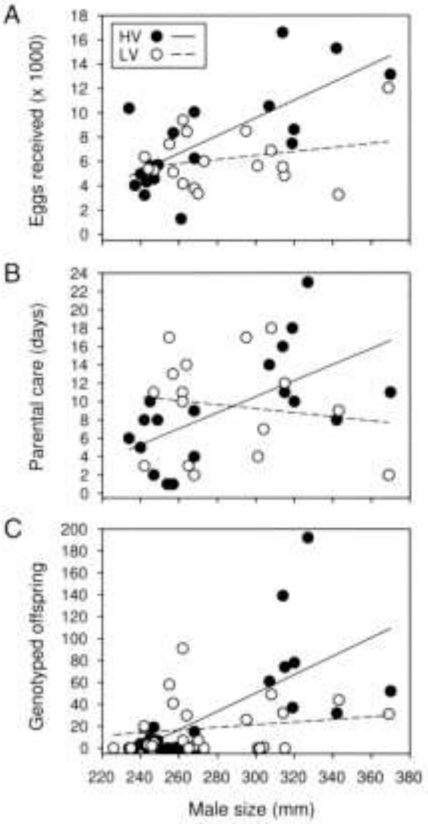

Fig. 1. (*A*) Number of eggs in individual largemouth bass nests of males with high vulnerability to angling (HV, full circles and solid lines) and low vulnerability to angling (LV, open circles and dashed lines), (*B*) duration (days) of parental care (starting at swim-up fry stage) provided by the nest-guarding males, and (*C*) number of genotyped fall recruits across nest-guarding males of different sizes.

are in agreement with earlier work assessing their vulnerability to angling using previous generations of HV and LV largemouth bass (19), indicating consistency of evolutionary differences among generations of fish selected for high and low vulnerability to fishing over three generations, respectively, despite no further selection over two subsequent generations. Finally, larger HV males provided parental care for a longer duration than any of the other males (i.e., smaller HV males, as well as larger and smaller LV males; Fig. 1*B*), as evidenced by the significant interaction between selection line and the covariate male size (Table 1). The shorter duration of parental care seen for the smaller males may reflect their expected lower energy reserves coming out of winter, thereby limiting their ability to sustain parental care activities, a situation that may be particularly acute for smaller HV males because of their higher metabolic rates (19–21). The lower willingness for smaller HV males, as well as smaller and larger LV males, to provide parental care may also be due in part to their lower mating success (i.e., fewer eggs received) after spawning (23). It is also possible that HV and LV males assessed the value of their current vs. future potential broods (29) differently. That is, HV males might have developed a higher level of expected mating success than LV males and as a result chose to abandon larger broods than did LV males.

When the experimental ponds were drained after the first summer growing season, in September 2009, a total of 11,375 juvenile (young-of-the-year) largemouth bass were recovered across the six ponds. Of the 1,200 offspring (200 per pond) randomly subsampled for parentage assignment, 1,189 (99.1%) were assigned to specific HV and LV male parents at a 95% confidence level. Of those 1,189 offspring, 740 (62%) were sired by HV males and 449 (38%) by LV males. The remaining 11 offspring were sired by a wild-type male that was initially mistyped as a female and remained undiscovered until observed guarding fry. Offspring sired by this fish were excluded from further analyses. Both the selected line (HV vs. LV) and size of the male had a significant interactive influence on individual reproductive success in terms of offspring numbers (Fig. 1*C* and Table 1). More specifically, the number of offspring produced by larger HV males was higher than the number of offspring produced by all other males (Fig. 2*C*). Comparison of total lengths of offspring also revealed that HV offspring (51 mm, SD ± 6.2) were, on average, 5% smaller than LV offspring (54 mm, SD ± 5.4) in fall (Table 1), consistent with earlier findings for juvenile HV and LV largemouth bass raised under food-limited pond conditions (20). It is likely that the higher metabolic rates of HV offspring were responsible for their smaller sizes in the present experiment. In addition, because all females used in this experiment were of wild-type origin (i.e., not selected for vulnerability to angling), the fact that our results still revealed an impact of elevated metabolic rates among the offspring of HV males suggests paternal inheritance of at least some metabolism-related aggressive traits (30). Another possible explanation, however, is that HV fish, despite having a higher growth capacity due to

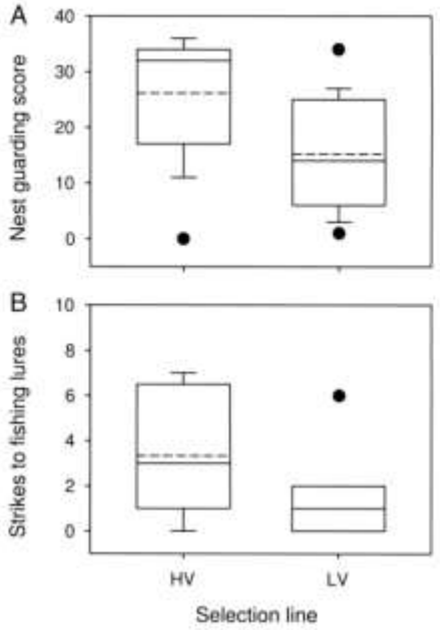

Fig. 2. (*A*) Nest-guarding intensity score and (*B*) aggression score (as measured by hits to fishing lures) for male largemouth bass guarding eggs with high vulnerability to angling lures (HV), and low vulnerability to angling lures (LV). Data are shown as box plot showing the median (solid line) and mean (dashed line).

elevated metabolic rates (21), did not ingest as many food items as LV fish owing to differences in their foraging ecology (31). Moreover, because it has also been shown that general swimming activity of HV and LV fish is similar in ponds (32), when food is limited, HV fish would be expected to pay the costs of increased metabolism and higher aggression, resulting in smaller size-at-age and potentially even an elevated size-dependent over-winter mortality (20, 33). As a result, the different metabolic rates of the HV and LV fish could result in relative survival probabilities for their offspring that vary depending on ecological context (e.g., high and low food availability and/or short or long growing seasons across latitudes). In summary, our findings provide empirical evidence that fish with different vulnerabilities to angling also exhibit differences in reproductive fitness and that in largemouth bass more-vulnerable fish indeed carry a higher fitness potential.

The higher relative fitness of the larger HV males can be attributed to several of their characteristics. First, the largest HV males had the highest mating success among all male classes, probably reflecting differential allocation of eggs via female mate choice, as previously described for a range of species in which male parental care offers key fitness benefits to females (27). The fact that the larger LV males did not receive as many eggs as the larger HV males suggests that compared with LV males, HV males court females more effectively and/or they are more attractive to them. Female mate choice may involve an expectation of the preferred males providing direct, material benefits (such as increased parental care for a female's offspring by more aggressive HV fish) or an anticipation of indirect, genetic benefits, as per the expectations of the "sexy-son" or "good-genes" hypotheses (34, 35). Even though the exact mechanism remains unknown, the result is an elevated reproductive fitness for HV males. Second, the HV males across all sizes were more active in providing parental care to their offspring (i.e., they spent more time fanning eggs and guarding the nest than LV males), and they were more aggressive toward potential predators, which likely contributed to their higher offspring numbers when ponds were drained. Third, the larger HV males provided the longest duration of parental care for the offspring (Fig. 2*A* and Table 1). The duration of parental care has been shown previously to be a key component in determining relative reproductive success among spawning male largemouth bass, reiterating the obligatory need for parental care to allow offspring survival (28).

Our results collectively suggest that in any given population, the largest, most aggressive males may have the greatest potential fitness, yet these males are also the ones that are most vulnerable to angling. As a result, largemouth bass populations subjected to angling harvest during the nesting phase can experience the selective removal of those individuals with the greatest potential for high reproductive success. Even in the absence of harvest, any temporary removal (e.g., an angling event in a catch-and-release fishery) could mean a loss of at least some fitness to egg predators (23, 24). As a result, in response to either harvest or catch-and-release angling, fish in exploited largemouth bass populations would generally be expected to evolve traits that reduce their exposure to fishing gear (i.e., behavioral and physiological characteristics similar to those observed for LV fish). Because those characteristics involve key behaviors that are involved in determining general levels of individual aggression, however, fisheries-induced selection would also be expected to evolutionarily alter parental care behaviors. The result could be a population-wide decrease in average parental care effort, including less time spent fanning eggs and reduced aggression toward brood predators, which could affect recruitment particularly in northern latitudes or populations with overall low productivity (36). The rate and amplitude of such an evolutionary change in these behavior/life history traits and their potential ramifications for recruitment (37) would of course depend upon the amount of harvest mortality, or for catch-and-release fisheries, the intensity of catch-and-release of male bass on nests. The resulting ecological implications for such an affected fish population would certainly be context dependent. That is, negative effects of this fisheries-induced evolution on population-level recruitment in the wild would be expected to be strongest in environments with abundant food resources (i.e., where LV are benefiting less from their lower metabolic rate), with short spawning seasons (i.e., where the opportunity to respawn after brood loss is limited, e.g., in northern latitudes), and with overall high egg predation potential (i.e., where the added wariness/timidity of LV-like fish would result in a greater reduction in fitness due to increased brood predation). In addition, fisheries-induced evolution to LV-like genotypes would almost certainly be accompanied by a concomitant decline in catchability on a population-level scale (18), a scenario that would be undesirable for recreational anglers that value catch rates (38).

Our study shows conclusively that fishing can target those individuals that exhibit the highest reproductive fitness potential, thereby establishing the potential for selection of behavioral traits that help a fish avoid capture, but in the process decrease its parental care abilities. For any species of fish with parental care that is targeted by recreational anglers, that evolutionary scenario may have consequences for the quality of the fishery (7) or for population-level recruitment (36, 37). Because some level of angling for nesting largemouth bass (both catch-and-harvest and catch-and-release) has been occurring in most, if not all, populations in North America for many years, we predict that many populations will show evidence for this fisheries-induced behavioral change; that is, they would already contain fish that are less vulnerable to capture than nonfished populations. Assessing the historical prevalence of such evolutionary changes through a controlled survey of vulnerabilities in the wild, coupled with reciprocal transplant studies using common garden experiments, would constitute a fascinating research approach for the future. If the changes in bass behavior and life history prove to be large enough to reduce recruitment, then management strategies designed to constrain or even remove the underlying selective pressure (angling nest-guarding male largemouth bass) may need to be implemented. Because a great proportion of recreational angling effort in North America is directed at species that provide some form of parental care (e.g., other Centrarchidae, members of the Ictaluridae) (22), the management implications of this study extend well beyond the largemouth bass.

Materials and Methods

The study was carried out at the Illinois Natural History Survey's Aquatic Research Facility at the University of Illinois at Urbana–Champaign, IL (40.07°N, 88.22°W) from April to September 2009, and animal use was approved by the Institutional Animal Care and Use Committee (protocol #10202). Males from two lines of largemouth bass that had previously been artificially selected to exhibit either high or low vulnerability to angling lures over three generations (18) were used as test subjects. Fish from the F5 lines, two generations after selection was halted, were used in the present work. Largemouth bass from both lines, which had been raised in ponds where they fed on natural food (e.g., *Erimyzon sucetta*, *Lepomis* spp., *Rana catesbeiana*), were recovered in April 2009 by draining the holding ponds. Male largemouth bass from the two lines were tagged individually with passive integrated transponder (PIT) tags and given a colored anchor tag inserted in the dorsal musculature to allow visual identification of individuals. After tagging, six replicate 2,500-m^2 earthen ponds were each stocked with four 3-y-old mature males from each line; male size [HV = 277 ± 36 mm total length (TL), mean ± SD; LV = 278 ± 39 mm TL] did not differ across lines (ANOVA $F_{1,41} = 0.01$, $P = 0.92$) or ponds (ANOVA $F_{5,41} = 0.95$, $P = 0.45$). A total of 42 unrelated wild females collected by boat electrofishing from Lake Mingo (71.7 ha), IL (40.21°N, 87.73°W) were implanted with PIT tags and stocked together with the HV and LV males. Genders were assigned according to the shape and size of their genital opening and the presence or absence of swollen genital papilla (39). Each of the six ponds containing male largemouth bass received seven females; female size (351 ± 68 mm TL) did not differ significantly across ponds (ANOVA, $F_{5,36} = 0.24$, $P = 0.94$). For subsequent genetic analyses fin clips were taken from all adults and preserved in vials containing 95%

ethanol. Each of the six ponds also received ~500 immature bluegill (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*) to serve as both potential egg predators and forage (23, 24, 33). These fish were obtained from the Kaskaskia Biological Station (38.71°N, 88.75°W) and a local hatchery [Little Grassy Fish Hatchery, Makanda, IL (37.39°N, 89.07°W)]. Sunfish from both sources were equally distributed across ponds.

After stocking of all fish, ponds were snorkeled every day for 6 wk beginning April 20, 2009, to locate males on nests and to identify the start of parental care (defined as the first day that a male largemouth bass was observed with eggs in his nest). To reduce disturbance to nest guarding fish, egg numbers were quantified by digitizing a method previously described for smallmouth and largemouth bass (40, 41). Digital photographs were taken from all discovered nests along with a ruler with 1-cm markings placed on the edge of a nest. Egg numbers were then quantified using the image manipulation software GIMP 2.6.12. All photos were overlaid with a digital grid of 1-cm^2 squares based on the 1-cm markings of the ruler. The total number of eggs was quantified by outlining the egg mass with a digital brush tool and counting the number of eggs in three of the 1-cm cells within the outlined egg mass, with one each from the center of the egg mass, midway to the edge, and the edge. The averages of these egg counts were used with the total number of squares within the outlined egg mass to calculate total egg numbers of nests, as adapted from refs. 40 and 41. Levels of parental care investment among the males of both lines (HV and LV) were assessed from shore by recording standardized nest-guarding behaviors every 10 s over a 2-min observation period between 7:30 AM and 10:00 AM, for a total of 12 distinct observations per individual. Assessments were performed when a male was seen on a nest containing eggs <2 d old. Observations started as soon as males became accustomed to the observer on the shore; i.e., they returned to their nests and resumed parental care activities. Largemouth bass that were positioned on their nest and fanned eggs during the 10-s observation period received a score of 3; males engaged in behaviors to deter nest predators during the observation period were given a score of 2; males simply swimming in proximity to the nest (<2 m) were given a score of 1. Males that remained >2 m from their nests during the 10-s observation period were considered absent from the nest and, therefore, received a score of zero. The highest possible nest guarding intensity score a male could achieve during the entire 2-min observation session was 36. As an additional metric of parental care intensity, the duration of parental care (in days) provided for swim-up fry was recorded for each individual male. Parental care was considered terminated when a male ceased to be observed in proximity to its developed fry. In addition, as an assessment of the aggression shown by males toward potential brood predators, the vulnerability to angling during the nest-guarding period was also quantified. The experimental angling protocol consisted of casting three different hookless fishing lures (a surface popper, a 6-cm white twister jig, and a 12-cm black plastic worm) to each male five times while they were guarding eggs (24). Hooks were removed from all lures so that males could not actually be captured, while permitting responses toward the lures to be quantified. The number of strikes at each lure was summed to generate a total aggression score, with a maximum of 15 strikes per angling session being possible (24). The response to angling by brood-guarding largemouth bass has previously been shown to be representative of nest defense behavior (23).

The six experimental ponds were drained between September 8 and 12, 2009, the total number of largemouth bass offspring determined, and a random subsample of 200 of those offspring collected from each pond. Each offspring was measured for TL, and a caudal fin clip for parentage analyses was collected as described above for the adults. Individual reproductive success of all HV and LV males, determined as the number of offspring produced per each male, was quantified by parentage assignment using microsatellite markers. Amplification of microsatellite DNA was performed by using a two-step multiplex PCR (42). Two sets of distinct DNA microsatellite markers taken from literature were used (43–46). "Multiplex 1" consisted of the microsatellites *MS13*, *MiSaTPW025*, *MiSaTPW038*, and *MiSaTPW068* and "Multiplex 2" of *Lma 10*, *Lma 21*, *Msa 18*, *MiSaTPW011*, and *MiSaTPW107*. DNA was extracted from fin clips by the use of a DNA isolation kit (PEQLAB Biotechnology) according to the manufacturer's protocol. PCR reactions were set up in 15-μL volumes containing 1.5 μL of DNA isolate. The master mix (13.5 μL) contained 3 μL Taq buffer, 1.5 μL (25 mM) $MgCl_2$ and 3 μL (1.25 mM) dNTP, adjusted primer amounts for the five microsatellites per multiplex, and 0.15 μL Taq polymerase (GoTaq Flexi DNA Polymerase; Promega). Sterile water was added for a total reaction volume of 15 μL. Annealing temperatures were optimized for each multiplex and amplifications performed on a Biometra T 3000 thermocycler. The reaction consisted of an initial 3-min denaturation step at 95 °C followed by 15 cycles of a 45-s annealing time (Multiplex 1 53 °C, Multiplex 2 58 °C) and a 30-s elongation period (72 °C) followed by 25 cycles with annealing times of 30 s, extension times of 15 s, and a final extension of 3 min. Microsatellite analysis was performed using fluorescently labeled forward primers (Sigma-Aldrich) and a capillary electrophoresis system (CEQ 8000; Beckmann Coulter). Detected fragments were scored using the CEQ 8000 fragment analysis software (Beckmann Coulter). Any allele resulting in multiple detection failures when performing the fragment analysis was excluded from the analysis and considered a null allele (47). The most likely parent pairs of an offspring were identified using the program CERVUS ver. 3.0 (48), where the typing error probability was set to a level of 0.01%. Candidate parents were determined by calculating the trio logarithm of the odds scores and assessing the trio confidence at a 95% confidence level. Offspring that were not assigned to a parent pair on a 95% confidence level were excluded from further analyses.

Statistical analyses comparing the two lines with respect to behavioral characteristics, mating success, relative reproductive success, and offspring size were subsequently performed using a complete randomized block design with multiple experimental units (male largemouth bass individuals) within a block (ponds) (49) using a mixed model procedure (SAS version 9.1; PROC GLIMMIX; SAS Institute; coding for the modeling approach taken from ref. 50). Ponds were considered to be blocks to account for interpond variance, and the selected line (HV, LV) entered as a fixed factor. To account for possible size-dependent contributions to individual reproductive success, male TL was used as a covariate in the model. To test for offspring size differences among paternal lines, offspring sizes were pooled for individual males, and resulting means were compared across lines using the approach described above. When comparing offspring sizes, the covariate male size was replaced by the day of spawning (starting with 1 for the first nest) to account for differences in offspring age. All initial models contained an interaction term between the main effect (selected line) and the covariate (size). Main effect by covariate interactions were removed from the final models when nonsignificant, indicating homogeneity of slopes (51).

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Paper IX

IX

Robert Arlinghaus, Josep Alós, Thomas Klefoth, Kate Laskowski, Christopher T. Monk, Shinnosuke Nakayama, Arne Schröder, 2016. Consumptive tourism causes timidity, rather than boldness, syndromes: A response to Geffroy et al. Trends in Ecology & Evolution, 31:92-94.

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Supplemental Information

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Letter

Consumptive Tourism Causes Timidity, Rather Than Boldness, Syndromes: A Response to Geffroy *et al.*

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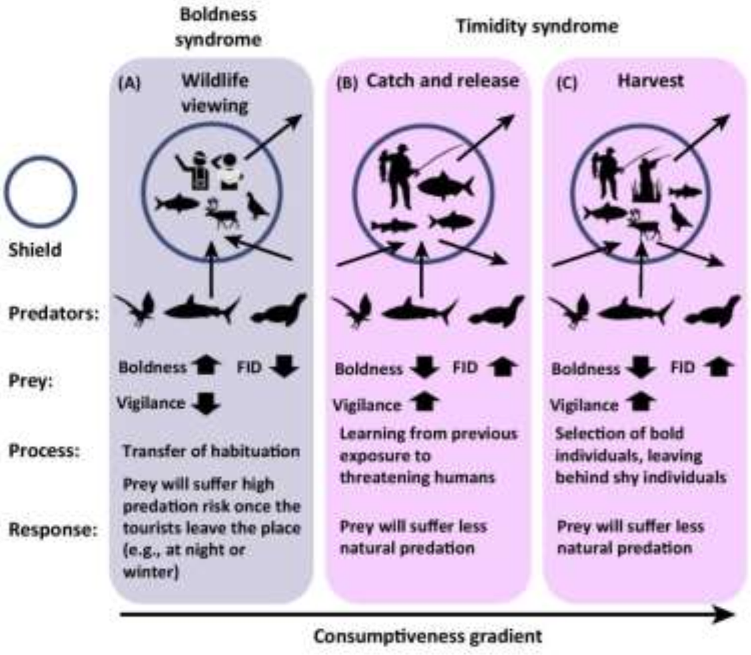
Geffroy *et al.* [1] proposed that nature-based tourism reduces the fearfulness and antipredator behavior of animals, leading towards a boldness syndrome that elevates natural predation rates and could trigger cascading effects on populations and communities. We agree with the framework, hypotheses, and future research needs proposed in [1], but they apply strictly to nonthreatening human–wildlife interactions. However, nature-based tourism is often consumptive, where wild-living animals are chased, stressed, and eventually harvested in activities such as recreational fishing and hunting. No threatening forms of human use of animals were elaborated in [1]. As a complementary perspective, we here propose that consumptive nature-based tourism might lead to opposite behavioral outcomes to those proposed in [1] by inducing a timidity, rather than a boldness, syndrome (Figure 1).

Human exploitation of wild-living animals creates a 'landscape of fear' [2,3]. A commonly reported plastic behavioral response of animals to human-induced predation risk involves increased antipredator behavior and heightened timidity, characterized by a greater use of refuges and reduced activity [2–8]. For such effects to happen, the experience of nonlethal, yet threatening stimuli caused by humans are often sufficient. For example, catch-and-release angling is increasingly common in tourism-based fishing operations. Being hooked, physiologically stressed, and eventually released promotes refuge-seeking behaviors that reduce vulnerability to fishing, which may also affect nonhooked conspecifics through social learning [8,9]. In addition to plastic effects within the behavioral reaction norm, lethal consumptive tourism may also cause evolutionary responses in a range of life-history and behavioural traits that collectively increase the average timidity levels of surviving individuals [3,6,7,10–12]. For example, bold, explorative, aggressive, and active behavioral types (aka 'personalities') within exploited wildlife populations are often selectively harvested [3,7,10–12]. The positive heritability characterizing most personality traits in turn could facilitate an evolutionary (i.e., genetic) response of timidity-related behaviors [6,7,12]. Increased timidity due to learning and/or evolutionary adaptation can occur in both predator and prey populations when they are exposed to threatening stimuli by recreational fishers or hunters. The net result for a prey species should generally involve a reduction, rather than an increase [1], in natural mortality risk because either the prey becomes shyer when they are exploited or it benefits from an increased timidity of the exploited predator, thereby being



Trends in Ecology & Evolution

Figure 1. Animals Respond to Human Presence Along a Gradient of Consumptiveness from Entirely Nonconsumptive Nature-Based Tourism to Harvesting-Based Exploitation (from Left to Right). When nonconsumptive and consumptive humans interact with wild animals, they create a temporal shield with different responses, including habituation (A) and fear of humans (B,C). (A) The original boldness syndrome introduced by [1]. The arrows represent the fluxes in-and-out of the prey organisms, once the humans leave the shield and the predators come in. In (A), a temporal shield between humans and animals is expected to induce higher boldness in the animals as a result of habituation to humans. When the humans leave the shield and predators enter, the prey is expected to be more vulnerable to natural predators as predicted by [1]. (B,C) The timidity syndrome introduced here. (B) A nonharvest shield that emerges when fishes exposed to catch-and-release fishing or wildlife exposed to hunting induce fear and antipredator behavior. The mechanism here is focused on learning in response to previous capture-and-release experiences or experience of shooting, possibly aggravated by social information flow. The flux of animals to the shield should be unidirectional because the animal density should remain fairly constant due to the lack of harvest. However, the fear of consumptive humans may in the long run also induce a migration outside of the shield if animals move into refuges, which might attract novel bold and explorative individuals to the shield, restarting the cycle. (C) The case when humans chase and harvest wild animals, where surviving individuals are bound to become shyer, increase their fearfulness and flight initiation distance (FID) in response to the behavioral-mediated selective process and through learning by private or public information. The surviving individuals then characterize a lower encounter probability not only with humans, but also in relation to natural predators, creating a temporal shield through a timidity syndrome. Animals might leave the shield to avoid the risk of death imposed by hunting or fishing, similar to (B), while naive bold and highly explorative individuals may occasionally enter from outside the shield because harvesting relaxes the density-dependent food competition and, hence, attracts migrants. The newcomers may then also be selectively harvested or plastically respond to the human predation risk. Similar to (A), fishers or hunters may also leave the shield in (B,C) due to seasonal patterns of exploitation, including holiday patterns, or because of constraints imposed by policy (e.g., a seasonal closure or a protected area). This would attract predators to the shields during the absence of humans, but prey might be less vulnerable to predation. Modified from [1] (A).

released from predation risk. However, in catch-and-release angling, released individuals might also suffer from immediate predation before even reaching refuges due to physical exhaustion and short-term behavioral alterations caused by stress.

The timidity syndrome proposed here in response to consumptive tourism not only predicts reduced natural mortality risks in prey organisms, but also implicates several other ecological and managerial consequences for social groups, populations, food webs, ecological services, and assessment of stocks. For example, animal groups might systematically lose keystone individuals and then show reduced ecological performance, such as during spawning migrations, where the leadership by old, experienced animals may be particularly important. Moreover, trophic relations are bound to be altered when hunted and fished animals are forced into refuges and away from profitable foraging sites. Thus, the timidity syndrome may moderate the degree of bottom-up or top-down control of food webs depending on the exact geographical configuration of foraging and refuge sites in an ecosystem [2]. Finally, from a social and economic perspective, increased ability to avoid capture (a key component and indicator of the timidity syndrome that we propose [5–9]) may not only lower the satisfaction of anglers and hunters by reducing catch rates or sightings, but also contribute to systematic erosion of the quality of user-dependent monitoring data as a surrogate of stock abundance due to hyperdepletion effects where catch rates or sightings decline more strongly than underlying abundance [6]. Reduced exposure to fish and wildlife due to increased timidity may also foster a belief among tourists that there is less wildlife or fewer fish than desired, which either threatens the economic operation by reducing demand or increases stakeholder conflicts.

Similar to the boldness syndrome [1], one of the most important outstanding questions in relation to the timidity syndrome is investigating whether the reduced exposure to fishing gear or hunters observed in heavily exploited stocks also alters the natural behavior of the exploited species in relation to natural predators. If fish and wildlife only selectively responded to human threat signals in the short term, the ecological and food-web effects of the timidity syndrome could be small and only socioeconomic impacts would be prominent. To sort this and other pertinent questions out, improved collaboration among field behavioral ecologists, fisheries and wildlife biologists, managers, and tourism operators is needed to investigate the propositions introduced here (Figure 1) and in [1]. Answering these questions is certainly not only of academic appeal, but also of large importance to fish and wildlife management as well as tourism.

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Letter

In Defense of the Ecotourism Shield: A Response to Geffroy *et al.*

Lee A. Fitzgerald[1,*] and Amanda L. Stronza[2]

Geffroy *et al.* [1] pose a bold scenario: nature tourists and ecotourists may create a 'human shield' in some circumstances, but ultimately tourism habituates animals and makes them susceptible to poaching and predation. In short, tourism is harmful to wildlife. The authors state that these problems from tourism can extend across entire populations of species and they suggest that tourism can even select for increased predation risk and drive loss of genetic diversity. We argue that these ideas are speculative and merit more careful analysis. We write with some urgency to critique the arguments and highlight the fallacies. We echo the authors' call for rigorous studies 'in the wild with real predators' and hope the paper will inspire many. In the meantime, asserting that nature tourism and ecotourism may be harmful to animals is premature and problematic. It sends a countervailing, mixed message to conservation stakeholders about the real importance of tourism for protecting wildlife, both in protected areas and in surrounding communities [2].

The shield of ecotourism extends across areas greater than the places where tourists interact with wildlife. When designed to provide benefits to conservation and local communities, ecotourism, sustainable tourism, and other forms of nature tourism help generate revenues and political support while also creating direct incentives to protect wildlife, ecosystems, and landscapes. The very foundation of the world's first national parks in the USA, and countless protected areas around the world, has been tourism. Tourism creates a large umbrella that is necessary for wildlife conservation and protection of large landscapes. Tourism helps fund programs for local villagers, who act as gatekeepers against poachers [3]. In Botswana, safari operators are leading efforts to reintroduce rhinos, bringing them in from areas where poaching is escalating. These animals are not habituated and most may never see an ecotourist [4]. Tourism has led to reduced hunting and transitioning from hunting to guiding, from direct, consumptive uses of biodiversity to indirect, less consumptive uses [5]. Buckley [3] noted that 'for over half of the red-listed mammal species at least five percent rely on tourism revenue to survive. For one in five – including rhinos, lions, and elephants – that rises to at least 15 percent of individuals. Simply put, if tourism money is cut abruptly, poaching will increase' [3].

The Geffroy *et al.* [1] model hinges on the transfer of habituation to the suite of predators in nature. To date, there is no conclusive evidence. The study on fox squirrels [6] cited as the most compelling showed that at one university and in suburban parks fox squirrels were less responsive to recordings of red-tailed hawks and coyotes. Because coyotes and hawks are scarce at the campus and parks, that study could not distinguish between transfer of habituation and low predation risk. Another study [7] found that in dik-diks habituation did not transfer to jackals. Likewise, predation on domestic cattle is an unconvincing example of how wildlife can become susceptible when habituated. The salient message from that

Paper X

X

Robert Arlinghaus, Kate Laskowski, Josep Alós, Thomas Klefoth, Christopher T. Monk, Shinnosuke Nakayama, Arne Schröder, 2016. Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish and Fisheries, 2016.

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Etymology of Ghoti

George Bernard Shaw (1856–1950), polymath, playwright, Nobel prize winner, and the most prolific letter writer in history, was an advocate of English spelling reform. He was reportedly fond of pointing out its absurdities by proving that 'fish' could be spelt 'ghoti'. That is: 'gh' as in 'rough', 'o' as in 'women' and 'ti' as in palatial.

Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications

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Abstract

Human exploitation of wild-living animals has been suggested to create a 'landscape of fear'. A consequence could be that individuals surviving intensive harvesting, either as a result of behavioural plasticity and/or evolutionary change, exhibit increased average timidity. In the aquatic world, such effects are particularly well documented in passively operated fishing gears common to many commercial and recreational fisheries, such as angling, trapping or gill netting. We thus propose that an exploitation-induced timidity syndrome should be a widespread pattern in fisheries. Importantly, we argue that the syndrome can be associated with several ecological and managerial consequences for social groups, populations, food webs, fisheries and assessment of stocks. We suggest research priorities to deepen our understanding of how exploited fish populations behaviourally respond to harvesting.

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Introduction

Heritable behavioural traits might be under strong selection in capture fisheries (Uusi-Heikkilä *et al.* 2008) as the behaviour of fishes determines the ease with which individuals are captured (Løkkeborg *et al.* 2014). In addition, fishes plastically alter their behaviour to reduce vulnerability to capture (e.g. Raat 1985; Klefoth *et al.* 2013; Alós *et al.* 2015a,b). However, despite its introduction as a hypothesis in the mid-20th century (Miller 1957), the question of whether and to what degree fisheries lead to persistent behavioural change and what are its consequences remain largely unanswered (Heino and Godø 2002; Uusi-Heikkilä *et al.* 2008; Heino *et al.* 2015). Here, we argue that an exploitation-induced timidity syndrome may be widespread in fish populations exploited by passive gear (i.e. gear where the capture process depends on the active behavioural decision by the target individual, von Brandt 1984). Passively operated gears, such as long-lining, angling, trapping, pots or gill nets, are common in many commercial and recreational fisheries and have been shown to preferentially catch bold, aggressive, explorative or active individuals (e.g. Biro and Post 2008; Härkönen *et al.* 2014; Wilson *et al.* 2015). By contrast, active gears, such as trawls or purse seines, may preferentially capture bold and social individuals (Heino and Godø 2002; Diaz-Pauli *et al.* 2015), but considerably less empirical research is available for active as opposed to passive gears. Therefore, we focus on passive gear types for the remainder of this paper.

An exploitation-induced timidity syndrome caused by passive fishing gear is defined as the emergence of fish populations that are consistently (i.e. across ecological contexts and time) more timid when exploited compared to unexploited populations of the same species (Arlinghaus *et al.* 2016). Depending on the species and type of gear, a timidity syndrome may involve greater use of refuges, reduced activity and space use (home range), reduced exploration, decreased willingness to ingest bait, reduced encounters of gill nets or traps and reduced aggression towards lures. If the exploitation-induced timidity syndrome we propose indeed alters the mean and variance of behavioural traits in the exploited population and assuming behavioural changes towards gear translate to changing intraspecific and interspecific interactions, this could have far-reaching implications for ecological and community processes as well as fishing quality and management as elaborated below.

Evidence for fisheries-induced behavioural change and its mechanisms

Consistent among-individual variation in behaviour in animal populations, referred to as behavioural types or animal personality, is now well established (for reviews in fish, see Conrad *et al.* 2011; Mittelbach *et al.* 2014). Réale *et al.* (2007) proposed five personality axes, that is exploration, activity, sociability, aggression and boldness (any form of risk taking), which are often correlated with each other forming behavioural syndromes (Sih *et al.* 2004). Natural selection tends to promote and maintain variation in personality traits, inter alia due to fluctuating selection pressures (e.g. Nicolaus *et al.* 2016). Moreover, natural predation has been shown to foster the development and/or evolution of behavioural syndromes between aggression and boldness, thus further maintaining variation in these personality traits (Fig. 1; Bell and Sih 2007; Dingemanse *et al.* 2007). By contrast, human harvesting is expected to result in directional selection on behavioural traits, eroding variation and resulting in a consistent shifts towards increased timidity (Fig. 1; Alós *et al.* 2012; Arlinghaus *et al.* 2016). Increased boldness is possible too, but these effects seem to be constrained on harvesting being unrelated to size or other traits (Jørgensen and Holt 2013) or strongly focused on juveniles (K. Andersen *et al.* unpubl. data), both of which are rare in most wild fisheries. The behaviour of exploited fish populations may also be modified as an indirect response to harvesting selection operating on correlated life history or morphological traits, particularly body size (Biro and Post 2008; Uusi-Heikkilä *et al.* 2015).

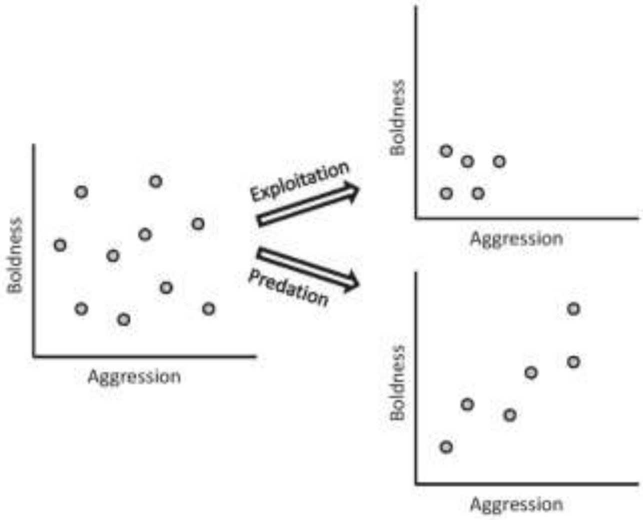

Figure 1 Phenotypic change induced by human exploitation and predation on a hypothetical population. Prior to exploitation or predation, individuals (grey circles) differ from each other in their levels of aggression and boldness. After exploitation, surviving individuals are on average less bold and less aggressive. After predation, however, surviving individuals converge on a behavioural syndrome where there is a positive correlation between an individual's level of aggression and their level of boldness as shown in several papers as a result of life-history trade-offs or genetic correlations (Bell and Sih 2007; Dingemanse *et al.* 2007). No such convergence is to be expected in the face of human exploitation, which happens to be directional.

In relation to passive gear, a fish's behaviour will affect two key processes: (i) encountering the gear and (ii) being retained by the gear. Spatial behavioural traits, such as swimming activity, exploration and the degree of space use, should affect the encounter rate (Rudstam *et al.* 1984; Alós *et al.* 2012). Indeed, there are a few examples that high swimming activity (Biro and Post 2008) and particularly exploration (Härkönen *et al.* 2014, 2016) increase the probability of capture in some passive fisheries. However, several recent studies failed to find a clear signature of fishing-induced selection on swimming activity, suggesting instead that other behaviours (e.g. the temporal dimension of space use or fine-scale attack behaviour in front of hooks) might be more important determinants of capture in selected fishes than activity per se (Binder *et al.* 2012; Olsen *et al.* 2012; Kekäläinen *et al.* 2014; Matthias *et al.* 2014; Bouletreau *et al.* 2016; Härkönen *et al.* 2016; Vainikka *et al.* 2016). There is also evidence from several fish species that individuals with larger space use are more vulnerable to capture by passive gear fishing (pearly razorfish *Xyrichtys novacula*, Labridae: J. Alós *et al.* unpublished data; northern pike *Esox lucius*, Esoxidae: Pieterek *et al.* 2016; negative findings Olsen *et al.* 2012 and C. Monk *et al.* unpublished data), suggesting that emergent spatial behavioural traits rather than activity per se can be under selection under certain situations (Alós *et al.* 2012; Matthias *et al.* 2014). In addition, there is increasingly firm evidence that both traps and angling remove aggressive and/or bolder individuals (which may or may not be more active), leaving behind stocks whose individuals are less aggressive and more timid (Wilson *et al.* 1993; Redpath *et al.* 2010; Sutter *et al.* 2012; Klefoth *et al.* 2012; Biro and Sampson 2015; Diaz-Pauli *et al.* 2015; Wilson *et al.* 2015; but see Wilson *et al.* 2011 for an exception). Although there is a need to more clearly identify the types of personality traits under selection in angling, overall increasing evidence suggests that in many situations behavioural traits will be under selection from passive gears and that in many situations selection operates on boldness or correlated traits such as aggression.

An evolutionary response to selection requires that the trait under selection be at least partly heritable. Philipp *et al.* (2009) documented that the composite phenotype of 'angling vulnerability' was indeed heritable ($h^2 = 0.15$), which corroborates more recent research documenting a large heritable component to most behavioural traits (Dochtermann *et al.* 2015). There is also considerable evidence across a number of species showing subpopulation-specific differences in catchability to angling when subpopulations were tested in common garden angling experiments (Beukema 1969; Brauhn and Kincaid 1982; Raat 1985; Dwyer 1990; Nuhfer and Alexander 1994; Garrett 2002; Klefoth *et al.* 2012). This work also suggests that the behaviours under selection by passive fishing gears have a genetic basis to allow fisheries-induced evolution of behaviour.

Importantly, what matters in an ecological and fisheries context is phenotypic, not genotypic change *per se* (Palkovacs *et al.* 2012), and here, a fish's ability to plastically adjust its behaviour to risk becomes important. There is abundant evidence that exploited fishes adjust their behaviour in response to human exploitation, regularly becoming more timid (Beukema 1969, 1970a,b; Askey *et al.* 2006; Januchowski-Hartley *et al.* 2011; Klefoth *et al.* 2013; Alós *et al.* 2015a,b; Philipp *et al.* 2015; Bergseth *et al.* 2016; Colefax *et al.* 2016; Tsuboi *et al.* 2016). A prime example are catch-and-release fisheries where fish become harder to catch as they increase their use of refuges and reduce their use of the open-water column (Yoneyama *et al.* 1996; Young and Hayes 2004; Askey *et al.* 2006; Klefoth *et al.* 2008, 2011, 2012; Baktoft *et al.* 2013). This response appears to be strongly driven by developmental plasticity and learning mechanisms (Fernö and Huse 1983), but in addition, cognition may itself show heritable variation and be selected upon by fisheries.

Ecological and managerial consequences of increased timidity

If populations exploited by passive gear are indeed systematically more timid than unexploited populations, this could have far-reaching consequences for social, population and community dynamics (Sih *et al.* 2011; Palkovacs *et al.* 2012) and the sustainability of fisheries. Next, we outline potential consequences for social groups, populations, communities and fisheries and its management, acknowledging that in reality, the consequences may feedback on each other as alluded to in Fig. 2.

Social consequences

We propose that the exploitation-induced timidity syndrome may lead to decreased group performance by impairing social behaviour through at least three mechanisms (Fig. 3a).

First, removal of bolder individuals may lead to a loss of collective information. When older and larger individuals are bolder through lower predation risk (Brown *et al.* 2007), selectively removing these individuals may leave behind a group composed of younger, less experienced individuals, reducing the quality of collective information. For example,

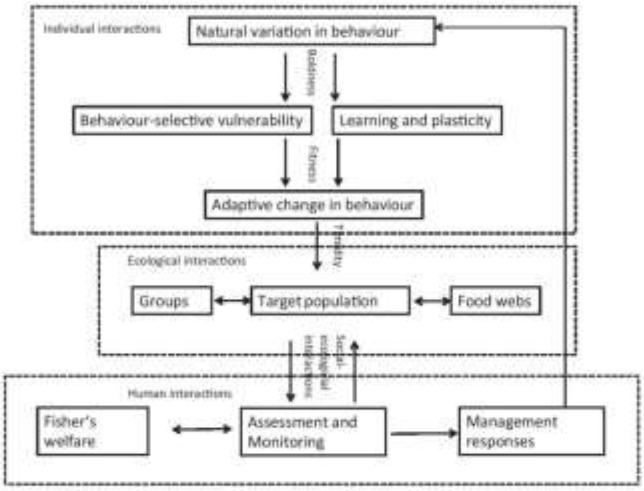

Figure 2 Conceptual diagram that outlines how changes at the level of the timidity of individual fishes feed back to affect ecological and in turn human interactions. Arrows ending in a box are meant to affect all elements in the box.

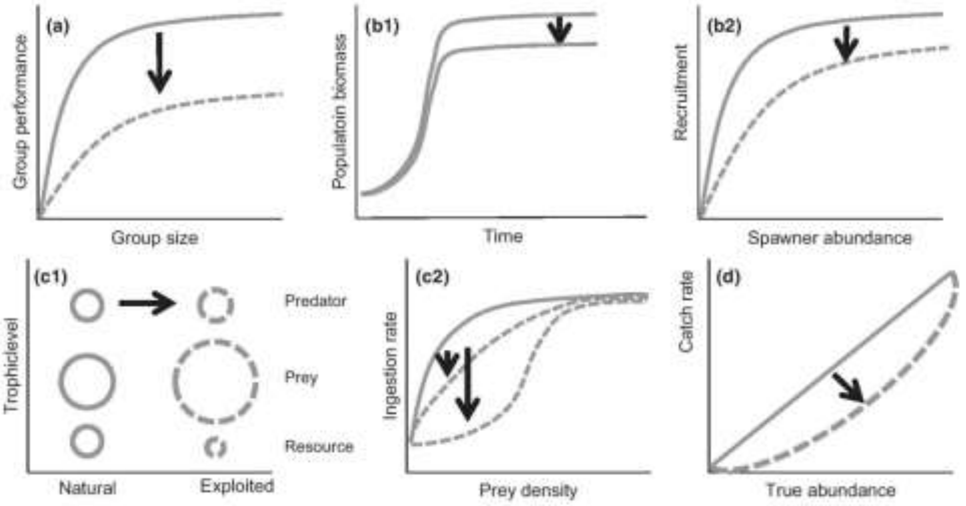

Figure 3 A schematic overview of the key consequences of exploitation-induced timidity syndromes (black arrows = before and dashed lines = after exploitation) for (a) social groups, (b) populations (b1 = changes in carrying capacity, b2 = stock and recruitment), (c) food webs (c1 = trophic cascades, c2 = functional responses) and (d) fisheries management. Note that these are prototypical expectations that may well be modified depending on ecological context and not always be expressed the way they are shown here, in particular for population and community levels (see text for details).

removing older individuals from a group can lead to rapid change in migratory patterns (De Luca *et al.* 2014), which may have been the case in Californian sardine (*Sardinops sagax*, Clupeidae) ceasing migration in the 1950s after overharvesting older and larger individuals (Petitgas *et al.* 2010).

Second, removing bolder individuals from a group could lower the accuracy in decision-making. Collective intelligence, often dubbed as the 'wisdom of the crowd', is widely seen in many social animals, and groups often outperform the decisions of single individuals even in the absence of well-informed individuals. Collective intelligence arises from offsetting ambiguity in information among individuals (Conradt and Roper 2003; Sumpter and Pratt 2009), and consequently, groups composed of diverse individuals may outperform groups composed of experts (Hong and Page 2004). Because boldness-selective fisheries could erode the diversity of personalities in a group, fishing might result in less diverse groups that are unable to make use of collective intelligence for vital decision-making regarding foraging and predator avoidance, for example.

Third, removal of bolder individuals might hamper behavioural coordination. Bolder individuals are more likely to use individual information compared to shyer ones (Webster and Laland 2011). This difference in information use leads to the specialization of social roles, such as leaders and followers in collective movement (Johnstone and Manica 2011). In stickleback (*Gasterosteus aculeatus*, Gasterosteidae), for example, differences in boldness between individuals reinforce social roles of leaders and followers through positive feedback (Harcourt *et al.* 2009). Shy individuals show low behavioural plasticity and struggle to take leader roles even when they are experimentally rewarded to do so, leading to decreased group performance (Nakayama *et al.* 2013). Therefore, selectively removing bolder individuals from the group might result in a leaderless group, with potential ramifications for fitness and population dynamics.

Population-level consequences

Population dynamics emerge from four processes, namely growth, recruitment, survival and dispersal. We acknowledge that dynamic interactions among these processes will ultimately determine the consequences of behavioural changes for population dynamics, but we will elaborate on potential consequences of a timidity syndrome for each of the four processes separately.

First, a key prediction of life-history evolution in response to fishing is the reduction in adult body size (i.e. downsizing, Jørgensen *et al.* 2007; Alós *et al.* 2014). In addition, passive gears tend to remove the fastest growing segment of the population (Biro and Post 2008; Saura *et al.* 2010; Alós *et al.* 2014; Crane *et al.* 2015; Evangelista *et al.* 2015; Pieterek et al. 2016). Hence, a timidity syndrome suggests an even stronger downsizing than expected from life-history evolution alone, which can ultimately affect population growth rate and biomass at carrying capacity (Fig. 3b1). Timidity could also incur a growth cost via trade-offs between survival and growth (Stamps 2007). Behaviours can also be linked with metabolism (Biro and Stamps 2008; Réale *et al.* 2010), which could have further effects on growth, possibly in counterintuitive ways (Cooke *et al.* 2007; Redpath *et al.* 2010; Hessenhauer *et al.* 2015). Depending on the local availability of food, less vulnerable individuals with lower metabolic rates may actually grow faster, and not slower, than their high-vulnerable, high metabolic rate, more aggressive conspecifics who under food limitation might not be able to turn their genetically higher growth capacity into rapid somatic growth (as shown in largemouth bass selected for vulnerability to angling, *Micropterus salmoides*, Centrachidae, Redpath *et al.* 2010; Sutter *et al.* 2012). The population dynamical consequences of behavioural selection associated with changes in growth might then be small or absent. Differentiating between competing hypotheses in relation to how selection on behaviour might affect growth is an important area for future research.

Second, foraging arena theory (FAT) predicts that risk-sensitive refuge use leads to Beverton–Holt-type recruitment (Walters and Juanes 1993; Walters and Korman 1999; Ahrens *et al.* 2012). Effects on recruitment may then be brought about or strengthened through increased risk-sensitive foraging of exploited, timid fish, shifting the stock–recruitment curve down (Fig. 3b2; Ahrens 1999). Beverton–Holt-type recruitment generally dampens population fluctuations and increases population stability (Ahrens *et al.* 2012). Risk-sensitive foraging in foraging arenas could also cause substantial delays in recovery of over-exploited populations, particularly in low-density environments (Walters and Juanes 1993). Fishing generally reduces the density of large adults, which can increase the relative and absolute densities of juveniles through relaxation of density-dependent reproduction and mortality (Schröder *et al.* 2009; Ohlberger *et al.* 2011). Such 'stage-specific positive mortality effects' (Schröder *et al.* 2014) may be reduced in strength or even disappear if behaviourally selective fishing generates more timid populations with lower reproductive output. Indeed, Sutter *et al.* (2012) found that high-vulnerable largemouth bass had higher offspring numbers and were preferred mating partners; therefore, removal of these individuals may reduce recruitment.

Third, in terms of natural survival, a clear-cut prediction from life-history evolution caused by fisheries is that natural mortality goes up with a faster life history (Jørgensen and Holt 2013). It is unclear whether the same result holds when fishing also selects on behaviour directly (a process not considered in Jørgensen and Holt 2013). In fact, a timidity syndrome might mean that fishes spend less time foraging and thus expose themselves less to risk. If exploitation reduces overall population sizes, this can increase resource availability and even timid fish could engage in short, but productive foraging bouts. In that case, natural mortality might not necessarily decline (Arlinghaus *et al.* 2016). In fact, bold, risky behaviour is often related to high natural mortality (Smith and Blumstein 2008), suggesting that timid fish might actually exhibit lower natural mortality levels.

Finally, timid individuals can have lower dispersal tendencies and movement distances than bolder individuals (Cote *et al.* 2010; Chapman *et al.* 2011), undertake shorter and fewer trips outside safe habitats or have smaller home ranges (Kobler *et al.* 2009; Harrison *et al.* 2015). By inducing timidity syndromes, fisheries may thus decrease the dispersal-based connectivity between local subpopulations. This could reduce the stability of metapopulations that rely on a balance of local extinction and re-colonization rates (Hanski 1998), alter source-sink dynamics as determined by the strength and direction of interhabitat movement of individuals (Àrneson *et al.* 2009; Casini *et al.* 2012) or slow down range expansions, impeding a population's scope for dealing with environmental change (Opdal and Jørgensen 2015).

Consequence for communities and food webs

Any changes to population dynamics or key traits in the population due to a timidity syndrome are bound to have spillover effects on communities, food webs and ecosystem functioning (Bolnick et al. 2011; Ahrens et al. 2012; Kuparinen et al. 2016). A timidity syndrome will influence an exploited population's interactions with other species and have potentially strong non-consumptive (Werner and Peacor 2003; Carpenter et al. 2010) and consumptive effects in addition to altering nutrient cycling (Palkovacs et al. 2012) (Fig. 3c1). Scaling up the effects of a timidity syndrome as we see it to communities and food webs is particularly complex and an area to highlight in need of more empirical research. Yet despite this complexity, whole-lake experiments have shown that predators can have strong non-consumptive effects on their prey that can exceed direct predation impacts and release lower trophic levels from predation control (Carpenter et al. 2010). The corollary of this argument would be that if predators themselves become timid in response to human predation threat, prey might be released from the 'cost of fear', which could have opposing effects than those described in the whole-lake bio-manipulation literature (reviewed in Carpenter et al. 2010).

A timidity syndrome in an exploited population that leads to weaker interactions among predator and prey has the potential to alter the functional response of survivors (Holling 1959) and thereby impact other trophic levels (Fig. 3c2). In simple food webs with strong interaction strengths, a reduction in overall feeding rate by a top predator on an intermediate consumer could have consequences on more basal trophic levels as the intermediate consumer is released from heavy predation pressure (Fig. 3c1; reviewed in Schmitz et al. 2004). Another plausible scenario emphasized in FAT is that more timid individuals may strongly reduce and occasionally even forgo feeding when resource availability is low and the risk of predation outweighs the benefits of feeding (Walters and Juanes 1993; Ahrens 1999). This could decouple predator and prey interactions and may then induce a sigmoidal type III functional response rather than the more typical type II (Pettorelli et al. 2015). Such changes would destabilize food webs (McCann et al. 1998; Fig. 3c2). Understanding the impact of increased timidity in a predator on their functional response through dedicated experiments is needed to sort out among the competing hypotheses.

Finally, because the timidity syndrome is expected to affect the habitat choice of exploited species and may alter metabolism (Redpath et al. 2010), it can also affect nutrient dynamics directly by influencing where nutrients are taken up, digested and excreted (Palkovacs et al. 2012) and indirectly through correlated changes in population-level metabolic rates (Hessenhauer et al. 2015). However, the predictions of what effects to expect are not clear cut. In the largemouth bass example, the low vulnerable phenotype showed lower basal metabolic rate (Redpath et al. 2010), but the same phenotype ingested more natural prey in mesocosm studies than the high-vulnerability conspecifics (Nannini et al. 2011). If the prey is digested in safe refuges by the low-vulnerable phenotypes, it will affect the distribution of nutrients, while the overall nutrient excreted may not necessarily change given the higher prey intake rate. More research in this area is certainly needed.

Consequences for fisheries and fisheries management

Consequences of a timidity syndrome for fisheries and fisheries management can be summarized in five areas.

First, the timidity syndrome allows the exploited population to compensate for fishing pressure. The adapted population can generally be expected to be more productive than the non-adaptive one in an exploited situation (Heino et al. 2013; Uusi-Heikkilä et al. 2015). However, a timidity syndrome and associated changes in life histories should also lead to a downsizing of adults (Jørgensen et al. 2007) and can thereby possibly lead to a decrease in yield (Matsumura et al. 2011). Increased refuge use and ability to avoid capture will also negatively affect catch rates of fishers (Philipp et al. 2009, 2015; Alós et al. 2015a,b; Tsuboi et al. 2016), with negative effects on fishing quality and fisher well-being (Arlinghaus et al. 2014; Beardmore et al. 2015).

Second, trophies are key determinants of satisfactory recreational experiences (Arlinghaus et al. 2014). The timidity syndrome should not only lead to fewer individuals reaching trophy size, but it will also reduce the exposure of these individuals to humans (Colefax et al. 2016; Tsuboi et al. 2016). Moreover, the positive size-dependency of capture probability may be disrupted as shown in a freshwater salmonid (Tsuboi et al. 2016). The slow disappearance of trophy animals may have social and economic consequences (Arlinghaus et al. 2016), in particular when trophies constitute an important component of the economic and social activity (e.g. trophy tourism angling). An alternative outlook could be that increased shyness reduces the exploitation probability of rare, highly desired specimens, increasing their general availability that fishers can, however, less and less enjoy due to reduced exposure to hooks (Tsuboi *et al.* 2016).

Third, a timidity syndrome might also reduce the effectiveness of traditional fisheries management tools such as protected areas (Bergseth *et al.* 2016). For example, if the propensity for long-distance fish migrations changes due to increased timidity, this might affect the optimal spacing of protected zones designed to maintain gene flow and meta-populations (Harrison *et al.* 2015). Alternatively, erosion of catchability could be a form of a natural harvest limit that works without putting harsh controls on fisher behaviour.

Fourth, the timidity syndrome promises to produce an evolving mismatch between catch rates and the true underlying abundance with important consequences for inferring the status of fish stocks (Alós *et al.* 2015a; Philipp *et al.* 2015) (Fig. 3d). Myers and Worm (2003), for example, suggested a large decline of top predator biomass in the oceans based on historical catch rates of passive gear. It is possible that over the long exploitation time, the surviving fishes evolved a lower vulnerability, which might have contributed to the drop in catch rates over time due to hyperdepletion effects (Alós *et al.* 2015a). Then, the status of the population or stock might look much worse than it actually is. Assessments that predict fewer fish in the water than are actually present could motivate ill-defined management responses such as the illegal introduction of fishes (Johnson *et al.* 2009) or the elevation of stocking to correspond with dissatisfied users that are unhappy with their low catches (van Poorten *et al.* 2011), which will put further pressures on the wild stock component (Post *et al.* 2002).

Finally, the timidity syndrome introduced here promotes behaviours along the bold–shy continuum that are adapted to an exploited environment but are maladaptive to an unexploited one (Uusi-Heikkilä *et al.* 2015). It is unclear whether and how fast population-wide levels of boldness can recover to pre-exploitation levels once exploitation ends. A key prediction of life-history evolution is that evolutionary change impairs full demographic recovery (Enberg *et al.* 2009; Heino *et al.* 2013; Uusi-Heikkilä *et al.* 2015), and it is likely that this is also the case in the timidity syndrome. In particular, if passive gears promote shy individuals, this might result in populations performing worse during interspecific competition (Colletér and Brown 2011; Briffa *et al.* 2015), thus decreasing the ability of an overfished population to rebound. Under some circumstances, when population numbers are driven too low, maladaptive behaviours can contribute to Allee effects, which might render that population unable to recover (Hutchings 2014). Further research is needed to understand whether the timidity syndrome aggravates issues already associated with the reduced abundance and altered size-structure at high fishing rates.

Implications for management and future research needs

The best approach to cope with the increasing timidity is promoting diversity of behavioural phenotypes (Fig. 2; Watters *et al.* 2003). Such diversity could be enhanced by the implementation of well-designed protected areas (Januchowski-Hartley *et al.* 2011; Alós *et al.* 2015a,b; Bergseth *et al.* 2016), fish relocation or stocking and in general mortality reductions. Properly designed protected areas should maintain and generate natural behavioural diversity, which can then spill over into exploited areas (Alós *et al.* 2015b). Where protected areas are infeasible, using stock enhancements or relocations of deliberately selected behavioural types could help maintain natural behavioural diversity. Another option is to change behavioural selectivity patterns by modifying size-based harvest regulations. Recent models suggest that harvest slots select for fast growth, rather than slow growth, and maintain high yield levels (Jørgensen *et al.* 2009; Matsumura *et al.* 2011) and more natural behaviours (Uusi-Heikkilä *et al.* 2015). Moreover, such regulations promise to maintain behavioural variation because it creates disruptive selection on key traits (Edeline *et al.* 2009).

The potential for fishing-induced selection on behaviour is perhaps well reasoned and increasingly documented. At the same time, there are many conflicting results on behaviour-selective fisheries involving laboratory tests of fish behaviour and there is a widespread lack of studies from the wild (Heino *et al.* 2015). For this reason, we stress that the various negative consequences of a potential timidity syndrome for groups, populations, communities, fishers and management outlined here must be considered reasoned hypotheses that demand proper research and testing. From the various areas mentioned above, probably the most direct effects of the timidity syndrome can be expected towards fisheries and fishing quality by altering catchability, but whether the timidity syndrome is widespread and affects natural ecological processes is an open question. It is well possible that exploited fishes only change their behaviour in relation to gear but not in relation to natural predation (Arlinghaus *et al.* 2016). It is therefore pertinent to improve our understanding of the degree to which a timidity syndrome is present in wild fish populations and what are the actual consequences.

To that end, we recommend a range of research areas that will help to understand how fish populations use space and time and the degree to which behaviour is plastic or under genetic control. The first research need is to better understand the heritability of key behaviours expressed by fishes in the wild. The second area is to improve assessments of fishing-induced selection and phenotypic change on behaviour, ideally conducted in the wild and in real fisheries. Documenting both heritability of traits and selection are necessary ingredients to demonstrate the potential for fishing to act as an evolutionary force on behaviour, but it is equally important to study phenotypic changes in behaviour caused by plasticity in the wild. Finally, there is a need to test the propositions of the timidity syndrome for social groups, populations, communities and food webs as well as fisheries. Both microcosms studies with small-bodied model organisms and work under natural conditions are needed in this respect. Ideally, experiments can be conducted targeting whole lakes or other replicated units (e.g. coral reefs or river sections) that allow entire communities to be assessed for their responses to behaviour-selective fisheries compared to controls. Such research will probably rely on cutting-edge telemetry approaches or other novel tools (e.g. underwater video cameras where feasible) to directly measure behaviour of animals *in situ*. Assessments of behaviour could then be coupled with assessment of ecosystem functioning and the tracking of offspring–parent relationships to understand fitness and ecosystem effects.

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