The effects of temperature and hatchery-rearing conditions on juvenile Atlantic cod (*Gadus morhua*) behavior

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90 ECTS thesis submitted in partial fulfillment of a
Magister Scientiarum degree in Biology

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I dedicate this thesis to the memory of my dear friend Xenios Christofi who left us too soon. You will continue to live in our hearts forever.

Declaration

I hereby declare that this thesis is based on my own observations, is written by me and has not been previously submitted, either in part or as a whole, at a postgraduate level.

Signature:

Acknowledgments

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Manuscript I- Disrupted behavior in hatchery reared juvenile Atlantic cod (*Gadus morhua*).

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General Introduction

Atlantic cod (*Gadus morhua*) is a demersal gadoid that has been one of the most important species in the North Atlantic fisheries for more than 500 years, with major economic, ecological and cultural significance (Cushing 1986; Kurlanski 1998). However, in the past few decades many of the cod stocks have declined dramatically, because of overfishing (Cook et al. 1997) and changes in the physical environment with major reorganizations of cod ecosystems (Frank et al. 2005). This in turn has lead to the rapid development of the cod farming industry.

Atlantic cod undergo four general life history stages; eggs, larvae, juveniles and adults inhabiting a number of habitats and a wide range of temperature regimes in each life stage (Lough 2004). Juvenile metamorphosis from larvae occurs in the water column, where juveniles remain for some months prior their settlement to the bottom (Fahay 1993). During their early juvenile life stage cod suffer from high mortality rates and behavioral flexibility regarding space use and antipredator tactics is crucial for its survival. At the point of settlement, juvenile cod become more associated to the bottom habitats and start demersal feeding (Lomond et al. 1998). Nearshore surveys have shown that higher densities of Atlantic cod juveniles are found in the northwest, north and northeast coast of Iceland (Palsson & Malmberg 1976; Astthorsson et al. 1994).

Benthic settlement is a period of high predation vulnerability for Atlantic cod juveniles while newly settled cod are susceptible to predation from a number of bathypelagic and demersal predators including adult cod (Connell & Jones 1991; Tupper & Boutilier 1995; Steele & Forrester 2002). However, complex benthic habitats can provide refuge and increase juvenile's survival, making habitats such as

cobble, kelp and sea grass beds beneficial to the early life history of the Atlantic cod development (Fraser et al. 1996; Gotceitas et al. 1997; Lindholm et al. 1999). In addition to seeking habitats that offer protection from predators, prey fish use several antipredator adaptations (i.e. morphological, physiological, behavioral) to reduce detection and be able to escape from predators (Lima & Dill 1990; Smith 1997; Lima 1998). The main strategies used by cod juveniles to reduce predator risk and prevent capture are; aggregating and hiding to minimize predator encounters and "freeze" or "fast start response" to escape predators (Gotceitas et al. 1995; Laurel & Brown 2006; Meager et al. 2006). Wild juveniles are able to fine tune those antipredator tactics based on their experience and learning, with cod juveniles mostly aggregating to reduce predation risk in the spatially "simple" pelagic environment. Moreover, juveniles express several other antipredation tactics such as staying at the bottom during daylight (Salvanes et al. 1994; Linehan et al. 2001) and using coloration that mimics the substrate (Lough et al. 1989).

In a changing environment, behavior allows an organism to adjust to either an internal or external stimuli and is the result of adaptations to environmental variables. Thus, behavior is constantly adapting through direct interaction with physical (e.g. temperature), chemical (e.g. oxygen) and social aspects of the environment to ensure maximum fitness and survival of an individual. Thus, Atlantic cod juveniles when shifting from pelagic to benthic life stage and while interacting with several biotic and abiotic (e.g. temperature) features of the environment in order to survive must be able to adapt to multiple different conditions using their behavioral flexibility and overcome possible fitness trade-offs.

Juvenile cod tolerate a wide range of temperatures compared to the other life history stages, with temperatures varying between populations (Lough 2004). Aquatic

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ecosystems are composed of several abiotic factors (e.g. light, salinity, current) with temperature being an all-pervasive attribute that limits the distribution, activity and survival of aquatic animals (Brett 1971). Temperature has both direct (e.g. physiology) and indirect (e.g. behavior) effects on fish and can thus alter the ecology and structure of fish populations. With the increased concern of global warming and the possible implications to marine fish, several articles and books have been published most of them examining temperature effects on fish physiology, metabolism and growth, swimming performance, reproduction and development with fewer studies regarding the effects of temperature on fish social behavior and possible effects on species and predator-prey interactions (Temple & Johnston 1997; Angilletta et al. 2003; Biro et al. 2007; Gilman et al. 2010).

Even though recently there has been an increased interest in cod juvenile ecology, more studies are needed to describe the life history, habitat characteristics and requirements of cod from the period of settlement especially in the nursery areas. Environmental changes are likely to have a rapid impact on animal behavior and behavioral plasticity can act to buffer the effect of environmental changes on survival, allowing adaptation to the environment (West-Eberhard 1989). In the theses we look at two factors that have been named as current threats to juvenile cod survival 1) hatchery reared cod and 2) changes in sea temperature. By examining the effects of environment on juvenile cod behavior we hope to clarify how these threats may affect wild cod populations.

In the first manuscript we examined the effects of hatchery-rearing environment on group behavior of age 0+ Atlantic cod juveniles. We tested wild and first generation of farmed fish in an experimental setting and we hypothesized that wild fish would be better at adjusting their behavior depending on experimental

environment (spatially "complex" or "simple") and conditions (competition from age 1+ juvenile and predatory cod) compared to farmed juveniles.

In the second manuscript we tested the effect of two temperatures, in both low and high ends of their tolerance limit, on social and feeding behavior of age 0+ Atlantic cod juveniles. We expected that activity, feeding attempts and willingness to forage in a risky environment will increase at higher temperatures to meet higher metabolic rates and energetic demands.

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Manuscript I

Disrupted behavior in hatchery reared juvenile Atlantic cod (*Gadus morhua*)

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Abstract

Early in their first year of life Atlantic cod (*Gadus morhua*) age 0+ juveniles undergo a transition from pelagic to bottom habitats and become demersal. Depending on habitat type, shoaling and hiding are two of the main strategies used by small fish to reduce predation risk. In the current study shoaling behavior of age 0+ wild and farmed Atlantic cod juveniles was examined in an experimental setting. Specifically, we tested if the process of rearing under aquaculture conditions affects the ability of juveniles to adjust shoal behavior according to the spatial complexity of the experimental environment. Trials were performed in the presence of a predatory cod, in the presence of an age 1+ juvenile and without older fish (control). Our results indicated that wild juveniles were more able to change their shoal behavior according to habitat, aggregating more closely in a simple environment but spreading out in the complex environment with shelters. In contrast, farmed juveniles did not vary their group behavior according to the spatial complexity of the environment. These findings suggest that hatchery-rearing does affect the development of shoaling behavior in age 0+ Atlantic cod juveniles and that hatchery-reared cod may be more vulnerable to predation than wild cod.

Keywords: Atlantic cod, *Gadus morhua*, shoaling behavior, group behavior, age 0+ juveniles

Introduction

The early life history of Atlantic cod (*Gadus morhua*) involves a habitat transition from the water column to the bottom and a consequent shift from pelagic to benthic prey (Lough et al. 1989; Lomond et al. 1998). Food availability and predation risk are the two main environmental factors that prompt the shift from the pelagic to the benthic habitat (Lough et al. 1989). Salvanes et al. (1994) showed, using the theory of optimal habitat shifts that by shifting to the benthic habitat rather than remaining to the pelagic, Atlantic cod juveniles would achieve a more favorable balance between survivorship and growth.

During their early transition from pelagic to benthic life age 0+ Atlantic cod juveniles, similar to the other demersal fish species, suffer high mortality rates (Biro et al. 2003a; Bystrom et al. 2003). This is expected as relatively naive individuals enter new habitats and encounter more experienced and opportunistic predators (Connell & Jones 1991; Tupper & Boutilier 1995b; Steele & Forrester 2002). Strong habitat associations are developed in juvenile Atlantic cod due to increased risk of predation compared to the other age classes, with cod juveniles preferring complex benthic habitats (i.e. cobble, kelp, sea grass) in the presence of a predator (Fraser et al. 1996; Gotceitas et al. 1997). Apart from higher risk of predation the age 0+ juvenile fish must quickly adjust to the more complex benthic environment that is already occupied by other small fish, invertebrates and age 1+ conspecifics. Previous studies, suggest competition for space and food both among and within these age classes of juvenile cod (Nordeide & Fossa 1992; van Duren & Glass 1992).

Habitat use by age 0+ juvenile cod is influenced by the bottom substrate type, predation risk and presence of conspesifics (Fraser et al. 1996). Studies examining the

effect of habitat in post-settlement juvenile cod survivorship have demonstrated that juveniles prefer complex benthic habitats (e.g. cobble-gravel, eelgrass) over spatially "simple" (e.g. sand) in the presence of both foraging and non foraging predator (Fraser et al. 1996; Gotceitas et al. 1997; Lindholm et al. 1999). Tupper & Boutilier (1995a, b) in nearshore field studies found that certain bottom habitat types are associated with the survival of age 0+ cod. More specific they found higher densities of juvenile cod in cobble and rocky substrates and this association has been attributed due to juveniles increased need to cover from predators.

Utilization of varied resourced should favor the evolution of behavioral flexibility, e.g. the ability of an individual to adjust to changes in the environments and respond appropriately depending environmental and social context (Stimson 1990; Juanes 2007). As in many animals several aspects of decision making in Atlantic cod juveniles are sensitive to the risk of predation and competition and therefore juveniles must compromise between predator avoidance and several other needs (e.g. foraging efficiency) (Lima & Dill 1990; Lima 1998; Metcalfe et al. 1999). Animals express a variety of defense responses to reduce predation risk but two main strategies are used by prey fish; shoaling and hiding (Krause & Ruxton 2002). The fitness benefits and costs differ between several anti-predator tactics and must therefore be optimized for each situation and can be fine-tuned based on experience (Lima & Dill 1990; Brown et al. 2006). Large aggregations are used by prey fish for avoiding detection and predation (e.g. Turner & Pitcher 1986; Magurran 1990), diluting individual risk (e.g. Morgan & Colgan 1987; Krause & Ruxton 2002) and confusing the predator (e.g. Krakauer 1995; Ruxton et al. 2007). Shelter can be used by prey fish mostly in bottom habitats, with Atlantic cod juveniles being able to avoid detection effectively through hiding (Gotceitas & Brown 1993; Gotceitas et al. 1995),

staying at the bottom during daylight (Koeller et al. 1986; Salvanes et al. 1994; Linehan et al. 2001) and by using coloration that mimics the substrate (Lough et al. 1989). Therefore, age 0+ cod juveniles must be able to adjust their antipredator behavior, during their transition from the pelagic to demersal life, and effectively use different strategies depending on the environment to minimize predation. Studies both in the field and laboratory have shown that wild age 0+ Atlantic cod juveniles aggregate more over unvegetated sand compared to over structured habitats with increased schooling behavior in the presence of a predatory cod (Laurel et al. 2004; Laurel & Brown 2006).

Animals that are reared in farm environments generally show less response to environmental changes in a suit of behaviors including feeding, predation avoidance and group behavior (Price 1999). From the time of hatching aquaculture conditions are likely to affect fish behavior both through learning and lack of environmental stimulation during development (Huntingford 2004). Consequently cultured fish often display poor survival in the wild in part because of behavioral modifications during domestication (Fleming et al. 2000; McGinnity et al. 2003). Behavioral differences between wild and farmed cod have been reported in previous studies showing that reared cod have different feeding behavior (Steingrund & Ferno 1997) and ineffective anti-predator behavior (Nødtvedt et al. 1999). Wild Atlantic cod juveniles are able to evaluate predation risk and respond, by sheltering (Gotceitas et al. 1995), shoaling (Grant & Brown 1998b) or decreasing activity (Nødtvedt et al. 1999). These responses seem to be reduced in farmed cod that are less competent at estimating risk and more active than similar sized wild fish (Svasand et al. 2000), making them more vulnerable to predation (Nordeide & Salvanes 1991; Nødtvedt et al. 1999). Recent studies have also shown that the early rearing environment affects the group behavior

of Atlantic cod juveniles. Atlantic cod juveniles from enriched rearing environment shoaled more tightly in an open tank, but were more spread out when there was rocky substrate. In contrast juvenile cod from plain rearing environment did not vary their group behavior in this way (Salvanes et al. 2007).

In the current study we examine the effect of hatchery-rearing on group behavior of age 0+ Atlantic cod juveniles. Specifically, we assessed juvenile aggregation and associations with larger conspecifics in wild and farmed 0+ juveniles in simple vs. spatially complex experimental environment. We hypothesized that wild fish would be better at adjusting their behavior depending on experimental environment and conditions compared to farmed juveniles.

Methods

Fish and maintenance

We used 120 age 0+ wild Atlantic cod juveniles (TL=9.15±1.46 cm) and 120 age 0+ farmed juveniles (TL=9.1±1.62 cm) in each experimental setting. We also used 10 age 1+ Atlantic cod juveniles (TL=20.27 ± 0.23 cm) and 4 age 3+ cod (TL= 48.86 ± 0.80 cm).

Both farmed and wild juveniles were obtained from a local land based juvenile cod farm (Gunnvör hf). Farmed fish were hatched from wild parents in March 2008. The fish were maintained in 8-10 kg/m^3 tanks with automatic feeding every hour and under a 12:12 h light-dark regime. The wild age 0+ juveniles were caught in September 2008 in several sites around Isafjordurdjup, Northwest Iceland (66º06'26.76"N, 25º53'17.50"W) period shortly after their transition to the more complex benthic life stage. Age 1+ and 3+ cod were of wild origin obtained from local sea cages.

At the experimental aquarium we used 6 tanks (100x110 cm and 30 cm) with salinity of 30-35ppt and temperature of ≈9ºC (Fig.1). Each tank was given a partial water change and cleaned every day. Fish were held under a 12:12 h light-dark regime and handfed daily to saturation with commercial pellets (size 2.5-3 mm). The experiments took place in October and November 2008.

Experimental protocol

Experiment 1: Aggregation of farmed and wild cod juveniles in a simple vs. spatially complex experimental environment (control treatment).

We observed fish aggregations in two test environments, spatially simple and spatially complex. The complex environment was constructed by placing stones (average size 15-20 cm) throughout the tank while the simple environment consisted of an empty tank (Fig.1). Groups of six fish from the same origin (wild or farmed) were randomly caught and transferred with hand-nets, released as a group into the test environment (100x110 cm and 30 cm) and allowed to acclimatize for 20 min prior the behavioral observations. Digital video was obtained by a remotely operated camera mounted 80 cm directly above each tank and continued for twenty minutes after the initial settlement period. Each individual was tested only once. Twenty groups of six wild fish (ten groups in each environment) and twenty groups of six farmed fish (ten groups in each environment) were tested (Fig.2).

Experiment 2: Aggregation of farmed and wild Atlantic cod juveniles with the presence of an age 1+ conspecific.

One age 1+ juvenile was transferred using hand-net and released into the test environment (both simple and complex) and allowed to settle. Groups of six fish from the same origin (wild or farmed) were randomly caught and transferred with hand-nets and released as a group into the test environment and allowed to settle for 20 min. Each individual was tested only once. The experiment was repeated in two types of test environment, spatially simple and complex. Digital video was obtained by a remotely operated camera mounted 80 cm directly above each tank and continued for twenty minutes after the initial settlement period. Twenty groups of six wild fish (ten groups in each environment) and twenty groups of six farmed fish (ten groups in each environment) were tested (Fig.3).

Experiment 3: Aggregation of farmed and wild Atlantic cod juveniles in the presence of a conspecific predator.

One age 3+ cod was transferred and released into the test environment (both simple and complex) and allowed to settle. Groups of six fish from the same origin (wild or farmed) were randomly caught and transferred with hand-nets and released as a group into the test environment and allowed to settle for 20 min. Digital video was obtained by a remotely operated camera mounted 80 cm directly above each tank and continued for twenty minutes after the initial settlement period. Twenty groups of six wild fish (ten groups in each environment) and twenty groups of six farmed fish (ten groups in each environment) were tested (Fig.4).

Video recording-Statistics

From the video recordings we took a series of pictures at intervals of 5,10,15,20 min. Distances between individual juvenile cod were measured using an open source image analysis software ImageJ version 1.42q available at (http://rsb.info.nih.gov/ij/) (Rasband 2009). We measured the distances between all six fish by using a point between the eyes as a reference point for each fish. We also measured the distance of each fish from the predatory cod (experiment 3) and from the age 1+ juvenile (experiment 2).

The total length (TL) of the each experimental fish was measured at the end of the experiment and compared across experiments using a t-test. Differences in group cohesion (as measured by the distances between the fish in a group) among substrate type and older conspecific presence was analysed using a linear mixed effects model repeated measures ANOVA. Habitat (complex or simple), treatment (control, with

age 1+ juvenile or with predatory cod) and origin (wild or farmed) formed the fixed effect factors of the linear mixed model. The random effect factor, accounted for repeated measurements of group of fish (trials) within different habitats and treatments. We also used a linear model with mixed effects to analyse the age 0+ juveniles inter- individual distances with respect to the distance between the age 0+ juveniles and the age 1+ juvenile or predatory cod. Again trials were used as a random factor with habitat (simple or complex), origin (wild or farmed) and treatment (with age 1+ juvenile or with predatory cod) as fixed factors.

We performed all our statistical analysis using R statistical program (version 2.9.2 The R Foundation for Statistical Computing, 2009)

Ethical Note

The experimental procedures had no obvious effects on the growth, health and survival rate of the fish used. Once placed into their experimental aquariums, fish were observed on a regular basis and predator present and aggressive acts were not seen to result in physical harm. Predatory cod used was raised from eggs in hatchery rearing conditions and fed with commercial pellets all his life. Prior the experiments predatory cod fed to saturation and the experimental tank used for our studies was relatively small (0.33 m^3), which made it difficult for the predatory cod to attack successfully (fast-start performance which requires space). To minimize stress, the predator was shown only 20 min in each trial. Aggressive interactions appeared in some of the groups of age 0+ juvenile cod but no physical damage occurred. No evidence of physical damage was seen on the fish during or after the experiments. All fish were held under appropriate water quality conditions and no fish was exposed to the predator stimulus more than once.

Results

Inter-individual distances

There were no differences in the length of experimental fish of either origin (t=1.43, p=0.18). Wild juveniles were 9.15±1.46 cm long, and farmed juveniles were 9.1±1.62 cm long. Our analysis showed significant interactions of origin (wild or farmed) ($F_{1,7179}$= 835.80, p<0.001), treatment (without an older conspecific, with a 1+ or older, predatory conspecific) ($F_{2,7179}$= 55.46, p<0.001), and habitat (simple or complex) ($F_{1,7179}$= 86.09, p<0.001) on age 0+ juvenile inter-individual distances. However further analysis showed that only wild age 0+ juvenile inter-individual distances significantly changed between habitats. Wild age 0+ juvenile cod changed their aggregation distances and aggregated more closely in the spatially simple environment and were more spread out in the spatially complex environment in all the experimental treatments; control ($F_{1,1189}$=61.28, p<0.001) with the age 1+ juvenile ($F_{1,1189}$=181.56, p<0.001) and with the predatory cod ($F_{1,1189}$=81.15, p<0.001) (Table 1) (Fig.5). In contrast the farmed age 0+ juveniles paradoxically aggregated more closely in the spatially complex environment when paired with predator ($F_{1,1189}$=13.55, p<0.001). A habitat effect on aggregation distances was not seen in experiments with no older conspecifics (control) ($F_{1,1189}$=0.003, p=0.95) or in the age 1+ juvenile treatments ($F_{1,1189}$=0.71, p=0.39) (Table 1) (Fig.5).

Age 0+ - age 1+ juvenile cod & Age 0+ - predatory cod distances

Our analyses of the distances between age 0+ juveniles and both age 1+ juveniles and predatory cod showed significant interactions of origin ($F_{1,1903}$= 24.69, p<0.001), treatment ($F_{1,1903}$= 6.98, p=0.008) and habitat ($F_{1,1903}$= 50.85, p<0.001). Further

analysis was applied showing that wild age 0+ juvenile cod maintained greater distances to a potential predator in a simple habitat than in a complex habitat. This applied to both age 1+ and predatory cod ($F_{1,469}=10.44$, p=0.0013 and $F_{1,469}=201.65$, p<<0.001, respectively) (Table 2) (Fig.6). In contrast the farmed juveniles showed no such response, neither in with age 1+ nor predatory cod ($F_{1,469}=2.37$, p=0.12 and $F_{1,469}=0.99$, p=0.31, respectively) (Table 2) (Fig.6).

Our analysis also showed that irrespective of habitat complexity, wild age 0+ juvenile stayed further away from the potential predator than they did from each other (for 1+ conspecifics as potential predators - $F_{1,829}=291.00$, p<0.001 and $F_{1,829}=291$, p=0.014 for simple and complex habitats, respectively; for older potential predators - $F_{1,829}=1308.74$, p<0.001 and $F_{1,829}=102.17$, p<0.001 for simple and complex habitats, respectively) (Fig.7). This effect was not seen in farmed juveniles (for 1+ conspecifics as potential predators - $F_{1,829}=0.80$, p=0.37 and $F_{1,829}=2.5$, p=0.11 for simple and complex habitats, respectively; for older potential predators - $F_{1,829}=0.09$, p=0.76 and $F_{1,829}=2.52$, p=0.11 for simple and complex habitats, respectively) (Fig. 8).

Discussion

In the present study we found significant differences in how wild and farmed origin juvenile Atlantic cod respond to environmental factors. The wild juveniles were found to respond to increased risk, e.g. open environments and predation threat, by aggregating more closely. In contrast the farmed origin juveniles did not alter their behavior in response to these environmental variables. Moreover, the wild age 0+ juveniles responded in a similar way to the presence of the age 1+ juvenile, indicating that perceived competition can significantly affect age 0+ juvenile cod behavior during benthic settlement. In contrast age 0+ farmed juveniles had similar distances within cohort and the older conspecifics with low levels of ability to perceived risk (Fig. 8).

Previous studies have shown that during the period of transition from pelagic to demersal life, age 0+ Atlantic cod juveniles become prey to numerous bathypelagic and demersal predators, cannibalistic adults and also face competition from other juvenile age classes (Koeller et al. 1986; Methven & Bajdik 1994; Fraser et al. 1996; Sogard 1997). Avoiding predation, finding and competing for food are monopolizing their daily routine. The survival of young fish depends in part on their ability to adjust their behavioral responses to changes in the environment, such as habitat complexity, predation risk and levels of competition. This behavioral flexibility is closely associated with early life experiences that influence the individual's behavioral repertoire (Huntingford 1993). Moreover, during the shifts from pelagic to benthic habitats an individual is exposed to novel experiences resulting in further behavioral modifications and a broader behavioral repertoire (Fuiman & Magurran 1994). Juvenile cod and in general juvenile life stage is characterized by high predation

pressure (Sogard 1997; Bailey & Duffy-Anderson 2001; Linehan et al. 2001). Prey fish use two main strategies for predator avoidance, hiding and shoaling (Krause & Ruxton 2002). During benthic settlement they are likely to change their antipredator tactic from predominantly shoaling to fleeing and hiding (Gotceitas et al. 1995; Laurel & Brown 2006). However, the effectiveness of these techniques post settlement is also dependent on the environment, for example substrate type (Gotceitas & Brown 1993; Anderson et al. 2007; Juanes 2007). Fish in structurally complex environments are commonly found to rely more in fleeing/hiding whereas fish in open environments shoal more frequently (Krause & Ruxton 2002). In our study the wild juvenile cod showed predictable responses to the shift between open and complex environments and predation. Our results showed that wild juveniles adjusted their shoaling behavior as they grouped more closely in all treatments in the simple test environment, but were more spread out in a spatially more complex environment with shelter places. Both open environments and predation risk increased wild juvenile aggregations (Fig. 5). In contrast, farmed juvenile fish were not able to adjust their shoaling behavior depending on the environment except, paradoxically, were more spread out in the simple than complex environment when paired with a predator. Farmed juvenile's distances within cohort were not significantly different compared with their distances from the age 1+ juvenile and predatory cod. Therefore, our result suggest that hatchery-reared cod are not able to estimate risk and respond by staying away from a potential predator while aggregating to reduce risk in the simple environment or taking refuge in the spatially complex environment and thus may be more vulnerable to predation compared to wild cod juveniles.

Various field studies have shown that age 0+ and age 1+ juvenile cod coexist in the same habitats (e.g. Methven & Bajdik 1994). Age 0+ juveniles suffer not only

from indirect high mortality rates due to competition from larger juvenile cod but also from cannibalism by age 1+ juveniles (Nordeide & Fossa 1992; Grant & Brown 1998a). Laboratory experiments found that age 0+ and age 1+ cod prefer the same habitats; and that age 0+ cod tend to avoid habitats preoccupied by age 1+ juveniles (Fraser et al. 1996). Our results are in line with these studies showing that wild age 0+ juveniles avoided older juveniles and aggregated more closely within cohort when an older juvenile was present. However, the farmed juveniles where on average found closer to the older juvenile than among cohort in the spatially simple habitat and in general the presence of an older juvenile did not alter their aggregation distances (Fig. 5, 8).

Recent studies have demonstrated that individuals reared in unnatural spatially "simple" environments and with limited learning experiences, have limited behavioral repertoire and learning ability (Olla et al. 1998; Brown & Laland 2001; Brown et al. 2003; Salvanes & Braithwaite 2005; Salvanes et al. 2007). Huntingford (2004) review paper includes several examples regarding the effects of nursery rearing environment on social, antipredator and feeding behavior in several hatchery fish species. The lack of responses to larger and potentially dangerous conspecifics and the lack of behavioral adjustments to increased environmental complexity seen in our study are in concurrence with previous studies that showed behavior differences between wild and farmed Atlantic cod juveniles due to the lack of experience in several aspects of their life (e.g. Steingrund & Ferno 1997; Nødtvedt et al. 1999).

Our results have implications for both industrial fish farming and conservation of wild cod. We find that juvenile Atlantic cod reared at industrial farming conditions may be more likely to suffer high mortality rates because of their lack of abilities to adjust their behavior to differences in the environment. Even though there are

differences in behavior between farmed and wild Atlantic cod juveniles numerous studies have shown that rearing environment affects the behavior flexibility and thus by enhancing the environmental variability in the rearing environment, farmed fish can increase their behavior repertoire (Nordeide & Fossa 1992; Salvanes & Braithwaite 2005; Salvanes et al. 2007). Further research on this and also other aspects of early juvenile behavior modification are needed for better understanding behavioral development in the early fish juvenile stages. In relation to developments in aquaculture our results highlight the necessity of paying special attention to behavior and varied experiences when raising fish for restocking purposes.

Acknowledgements

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Figures
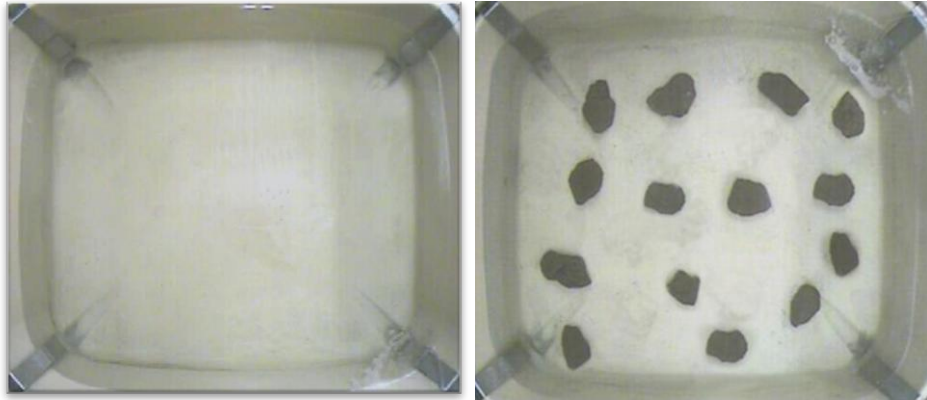
Figure 1. *Spatially simple and complex experimental environment.*
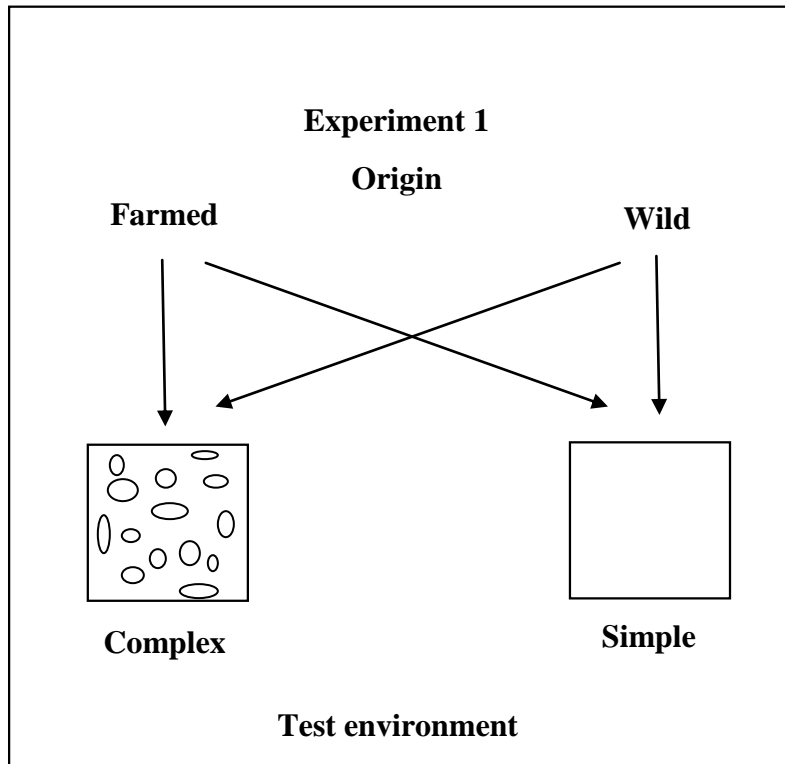
Figure 2. *Schematic figure of experiment 1. Ten groups of six farmed and wild juveniles were tested in either simple or complex test tank.*
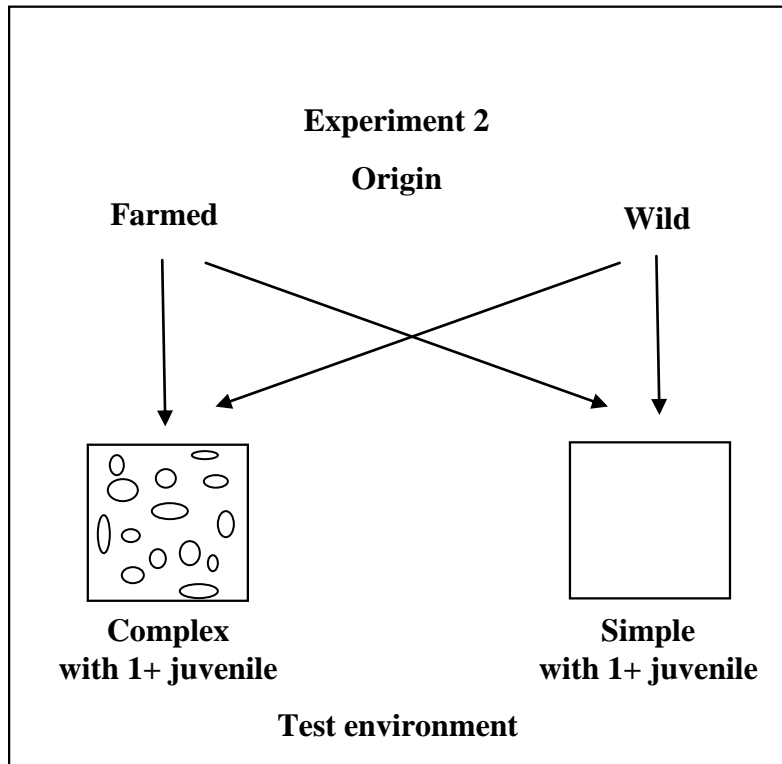
Figure 3*. Schematic figure of experiment 2. Ten groups of six farmed and wild juveniles were tested in either simple with 1+juvenile or complex with 1+ juvenile test tank.*
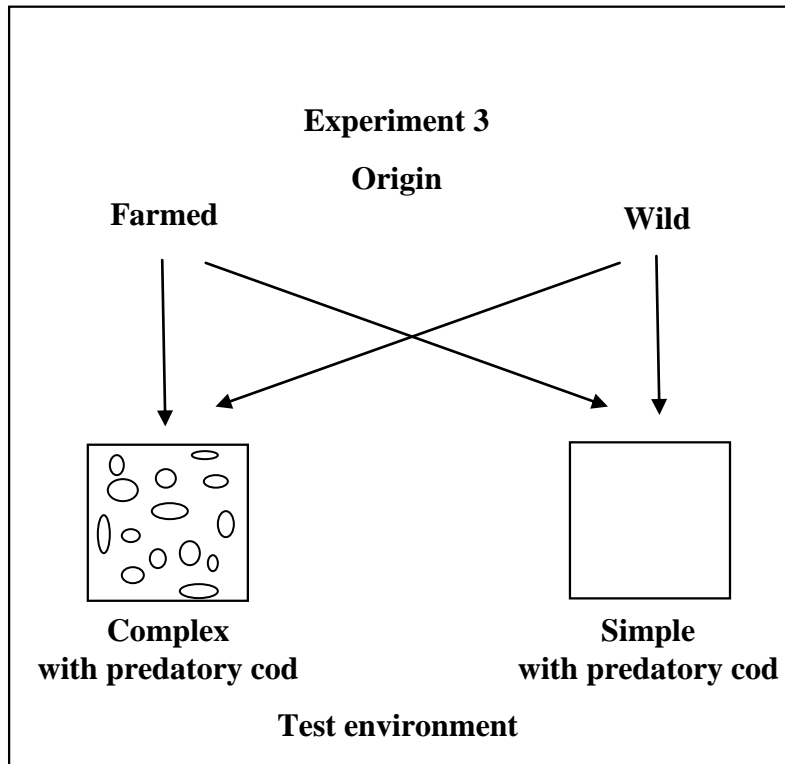
Figure 4. *Schematic figure of experiment 3. Ten groups of six farmed and wild juveniles were tested in either simple with a predatory cod or complex with a predatory cod test tank.*
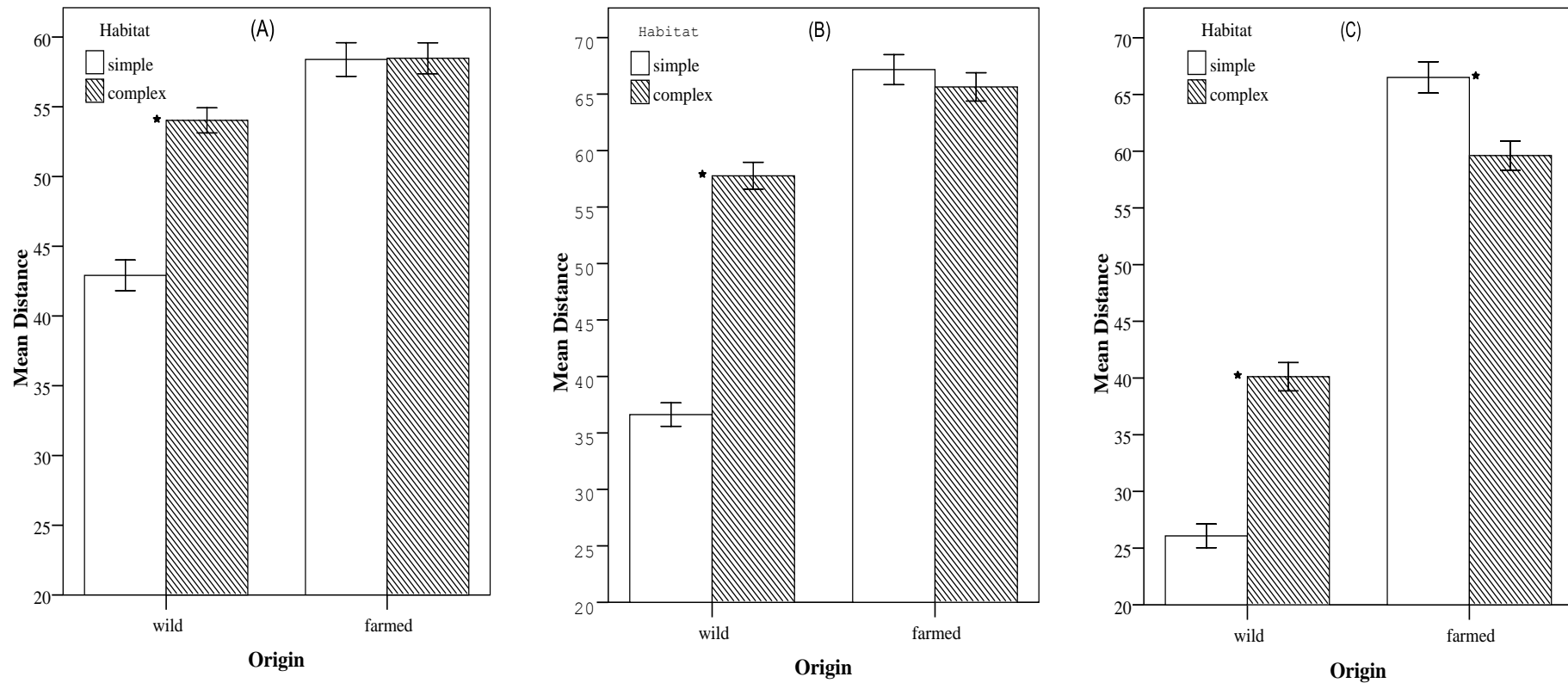
Figure 5. *Mean (±1 SE) distance (cm) among age 0+ juvenile cod. Age 0+ juvenile cod in the (A) control treatment (B) with age 1+ juvenile and (C) with predatory cod in the experimental tank. The habitat used was spatially simple and complex. * indicates a significant difference between the two habitats.*
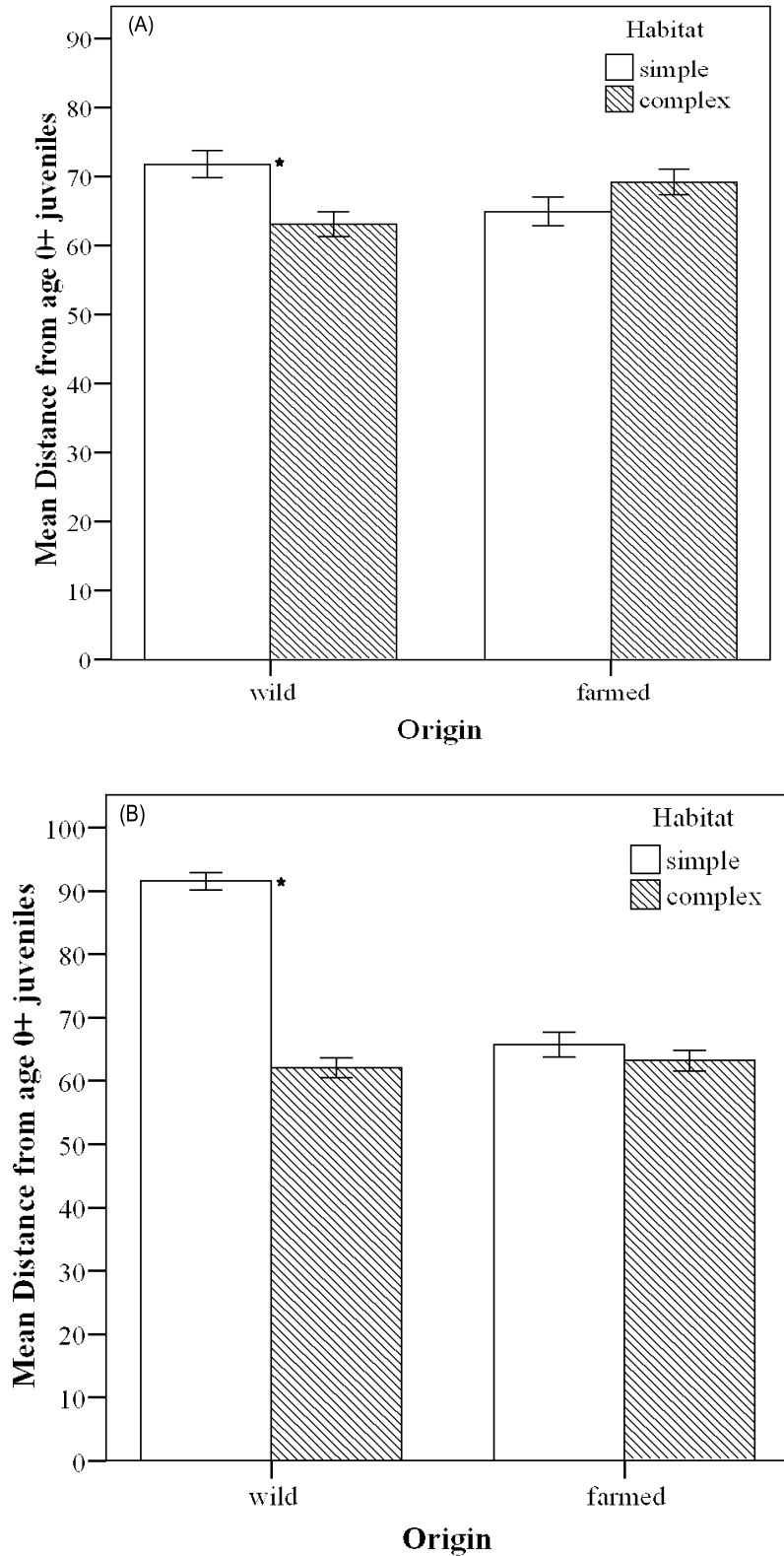
Figure 6. Mean (±1 SE) distance (cm) between (A) age 0+ juveniles and age 1+juvenile and (B) between age 0+ juveniles and predator. The habitat used was spatially simple and complex. * indicates a significant difference between the two habitats.
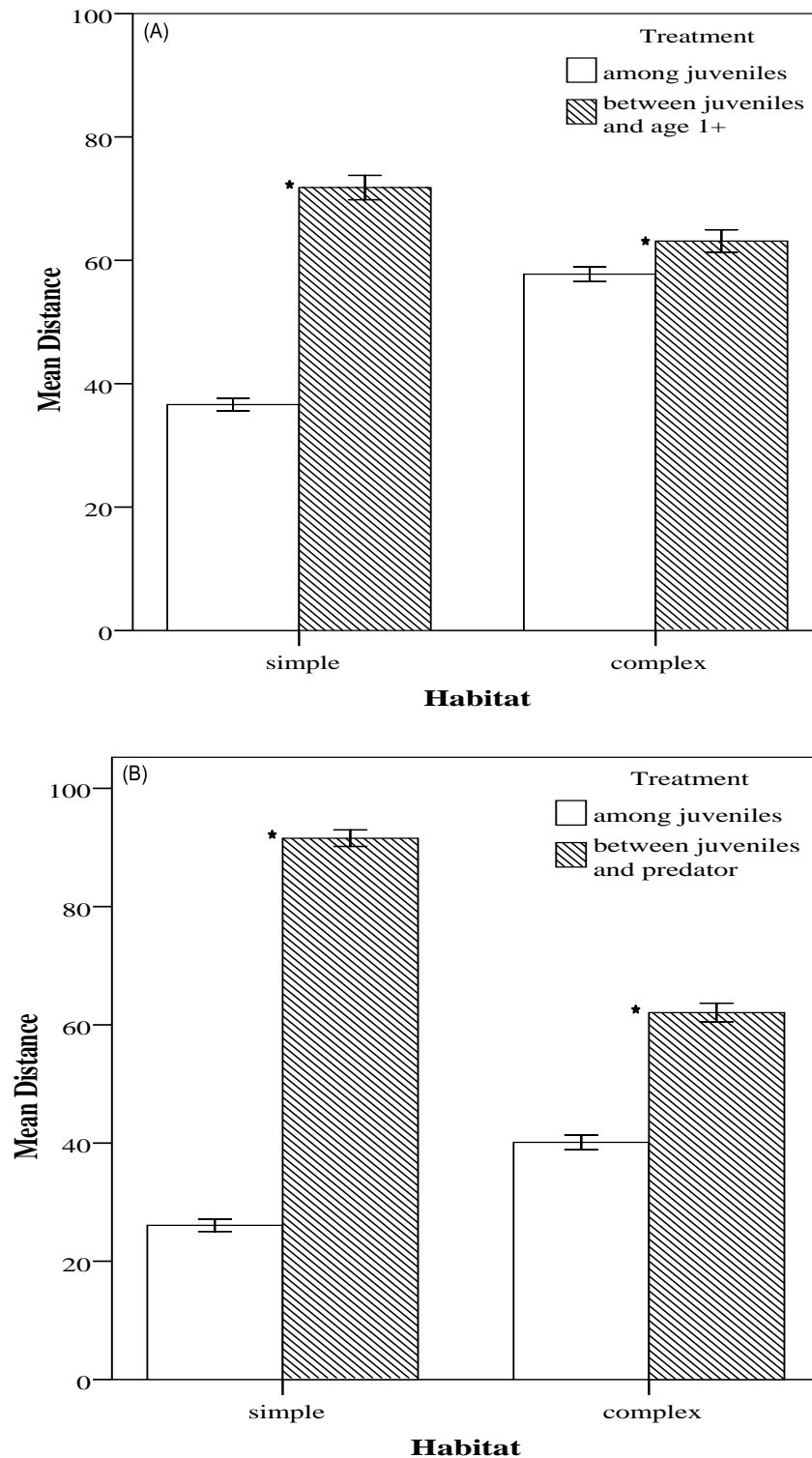
Figure 7. *Mean (±1 SE) distance (cm) among (A) wild age 0+ juveniles and between wild age 0+ juveniles and age 1+ juvenile, (B) wild age 0+ juveniles and between wild age 0+ juveniles and predator. The habitat used was spatially simple and complex. * indicates a significant difference between distances among juveniles and distances between juveniles and older conspecific.*
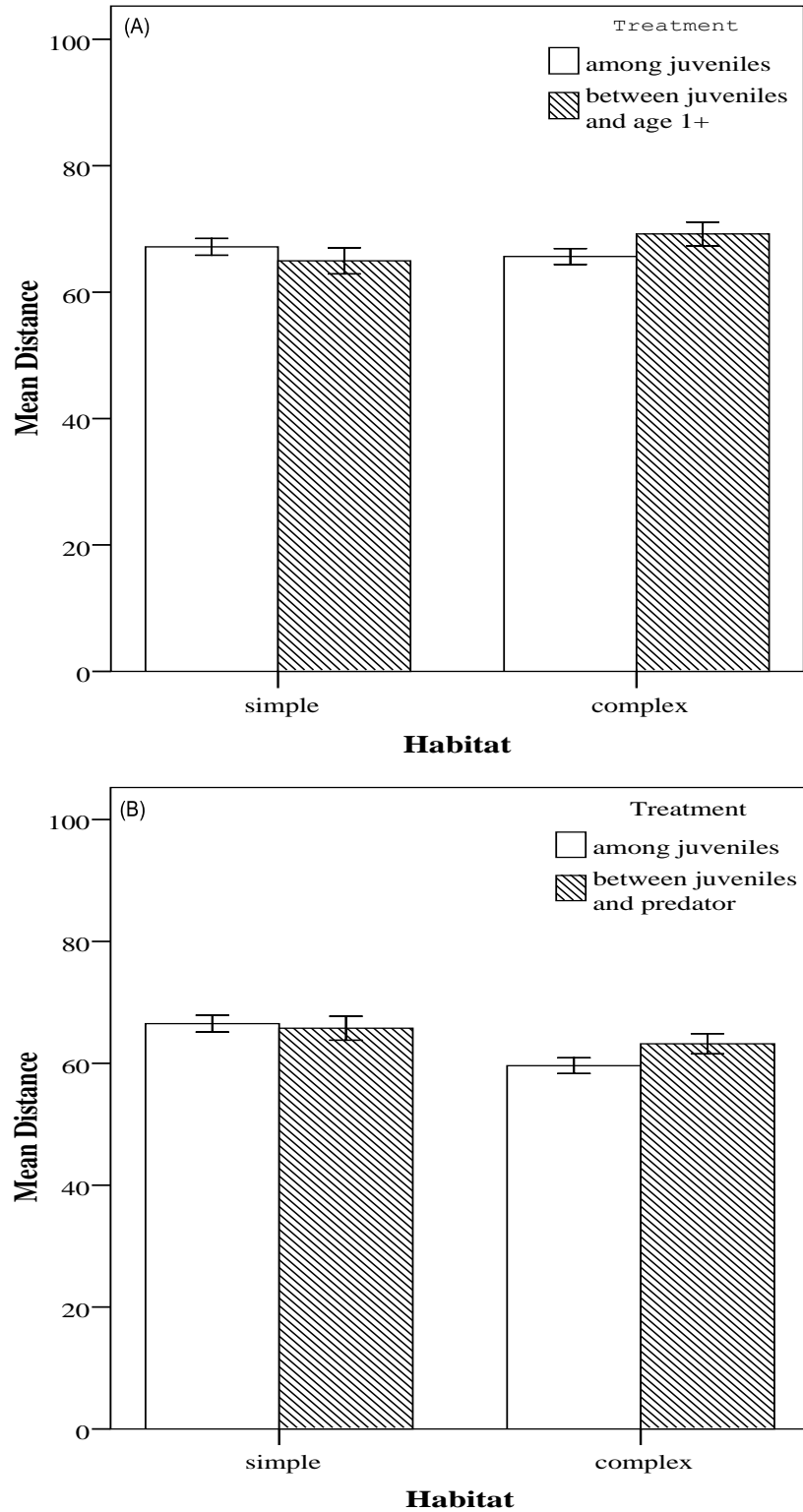
Figure 8. *Mean (±1 SE) distance (cm) among (A) farmed age 0+ juveniles and between farmed age 0+ juveniles and age 1+ juvenile, (B) farmed age 0+ juveniles and between farmed age 0+ juveniles and predator. The habitat used was spatially simple and complex. * indicates a significant difference between distances among juveniles and distances between juveniles and older conspecific.*

Tables

Table 1. *Analysis of variance of group cohesiveness of age 0+ juvenile Atlantic cod showing the effect of habitat complexity (spatially "simple" and spatially "complex"). F-values are given for all combinations of fish origin (wild and farmed) and treatments.*

	Treatment	Source	numDF	denDF	F-value	p-value
Wild	Control	Habitat	1	1189	61.28	< 0.001***
	With age 1+	Habitat	1	1189	181.56	< 0.001***
	With predator	Habitat	1	1189	81.15	< 0.001***
Farmed	Control	Habitat	1	1189	0.0032	0.95
	With age 1+	Habitat	1	1189	0.71	0.39
	With predator	Habitat	1	1189	13.55	< 0.001***

Table 2. *Analysis of variance of distances between age 0+ juvenile Atlantic cod and older, potentially threatening conspecifics, showing the effect of habitat complexity (spatially "simple" and spatially "complex"). F-values are given for all combinations of fish origin (wild and farmed) and threat treatments.*

	Treatment	Source	numDF	denDF	F-value	*p*-value
Wild	With age 1+	Habitat	1	469	10.44	0.0013***
	With predator	Habitat	1	469	201.65	< 0.001***
Farmed	With age 1+	Habitat	1	469	2.37	0.12
	With predator	Habitat	1	469	0.99	0.31

Appendices

Table 1. *Analysis of variance of age 0+ juvenile aggregations compared between habitats (spatially "simple" and spatially "complex"), treatments and origin.*

	Source	nmDF	denDF	F-value	p-value
F	(Intercept)	1	7179	7784.328	<.001
	Habitat	1	7179	86.098	<.001
	Origin	1	7179	835.805	<.001
	Treatment	2	7179	55.466	<.001
	Habitat:Origin	1	7179	178.289	<.001
	Habitat:Treatment	2	7179	7.250	7e-04
	Origin:Treatment	2	7179	71.774	<.001
	Habitat:Origin:Treatment	2	7179	7.083	0

Table 2. *Analysis of variance of age 0+ juvenile distances from either age 1+ juvenile and predatory cod compared between habitats (spatially "simple" and spatially "complex"), treatments and origin.*

	Source	nmDF	denDF	F-value	p-value
F	(Intercept)	1	1903	11473.289	<.001
	Habitat	1	1903	50.857	<.001
	Origin	1	1903	24.690	<.001
	Treatment	1	1903	6.982	0.0083
	Habitat:Origin	1	1903	60.844	<.001
	Habitat:Treatment	1	1903	29.186	<.001
	Origin:Treatment	1	1903	21.889	<.001
	Habitat:Origin:Treatment	1	1903	7.523	0

Manuscript II

Reaching the limit: reduced behavioral flexibility of juvenile Atlantic cod (*Gadus morhua*) at higher temperatures

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Abstract

Temperature affects fish physiology and thus several aspects of fish behavior. However knowledge on the effects of temperature on Atlantic cod juvenile behavior is limited. In the present study age 0+ Atlantic cod juveniles (*Gadus morhua*) were exposed to two different temperatures treatments within their thermal limit (3ºC-13ºC) and their behavioral responses (e.g. swimming activity, aggressiveness, aggregation) were observed. We also examined the effects of temperature in feeding motivation and time needed to recover from a simulated predator attack. As anticipated juveniles held at the temperature of 13ºC were more active with higher feeding motivation than juveniles held at 3ºC. Atlantic cod juveniles held at the temperature of 13ºC were more aggressive than juveniles held at 3ºC when food was placed in the centre of the experimental aquarium and needed less time to recover from a simulated predator attack and start their feeding attempts again. Juvenile cod held at 3ºC aggregated more and used sheltering in the feeding and added risk treatments. These results show that temperature has a significant effect on activity and several aspects of juvenile Atlantic cod behavior. In general, the juveniles responded less to simulated risk at 13°C. This suggests that at the upper end of their thermal tolerance juvenile Atlantic cod have diminished abilities to alter their behavior in response to environmental change, either because of consequential increase in activity or higher metabolic demands. Thus, our results suggest that with the expected increase in northern sea temperature, several aspects of juvenile cod behavior, related to feeding and predation vulnerability, are likely to be disrupted.

Keywords: Atlantic cod, *Gadus morhua*, temperature, behavior, activity, aggression

Introduction

Temperature is one of the most important environmental factors to influence the life of aquatic organisms (Brett 1971) and has significant affects on fish physiology and several aspects of fish behavior (Crawshaw & O'Connor 1997; Temple & Johnston 1997; Angilletta et al. 2003). The increasing northern sea temperature can therefore be expected to have both direct (e.g. physiology) and indirect (e.g. competition, predation) effects on fish populations (Biro et al. 2007; Portner & Farrell 2008; Gilman et al. 2010).

Temperature affects fish physiology through its effects on enzyme reaction rates (Hochachka & Somero 2002). Water temperature controls the metabolic efficiency for physiological functions such as food consumption, digestion, reproduction, activity and survival (Jobling 1994; Hillman et al. 1999). Previous studies have shown that when the water temperature increases, activity levels and especially swimming performance also increases to a limit and then declines with further increase of temperature resulting in a bell-shaped curve (Johnston & Ball 1997; Taylor et al. 1997). The same is true of maximum swimming speed and fish reaction (Roussel et al. 2004; Claireaux et al. 2006). Increase in water temperature within the animals tolerance limit initially leads to an increase in food consumption. At low temperatures because of low metabolic rates, food intake and, as a consequence, growth is restricted (Xie & Sun 1992; Jobling 1994). Through these physiological effects, temperature can affect the structure of aquatic communities, including survival, foraging as well as social behavior, species and predator – prey interactions (Dunson & Travis 1991; Fuiman & Batty 1994; Childs & Clarkson 1996; Domenici et al. 2007; Gilman et al. 2010).

By affecting activity, water temperature partly determines predation risk in many taxa, including fish. At higher temperature fish are required to spend more time actively foraging to locate their prey. Predator energetic demands are also increased and therefore prey fish mortality rates are more likely to be much higher at higher temperatures (Werner & Anholt 1993; Abrahams et al. 2007). Pauly (1980) showed in 175 different populations of 84 fish species, including Atlantic cod, that natural mortality is related to water temperature. Previous laboratory and field studies have shown that prey fish respond differently as they balance the increased demand of energy and risk of predation at higher temperatures.

Laboratory studies have shown that with an increase in water temperature, guppies (*Poecilia reticulata*) spent significantly more time schooling and inspecting in the presence of a predator, suggesting that water temperature can affect antipredator behavior in guppies (Weetman et al. 1998, 1999). Recent studies have also shown that water temperature affects boldness and aggressiveness in fish (Biro et al. 2007; Biro et al. 2010). Fish held at higher temperatures were more active, bolder and more aggressive. Studies on convict cichlids (*Cichlasoma nigrofasciatum*) and brook trout (*Salvelinus fontinalis*) showed that at warmer temperatures, fish were more aggressive and consumed more food (Ratnasabapathi et al. 1992; De Staso & Rahel 1994; Taniguchi et al. 1998). Biro et al. (2007) suggested that among age 0+ rainbow trout (*Oncorhynchus mykiss*) mortality was related to temperature due to greater risk taking to maintain growth rates at higher water temperatures.

Atlantic cod, *Gadus morhua*, is one of the most important fish species in the North Atlantic fisheries. Several studies have examined the effects of temperature on Atlantic cod, most of them in response to the potential climate change impacts in the north Atlantic ecosystems (Wood & McDonald 1997). Previous studies include the

effect of temperature on physiology and metabolism, (e.g. Claireaux et al. 1995; Claireaux et al. 2000), swimming performance (e.g. Winger et al. 2000; Peck et al. 2006; Guan et al. 2008), feeding and growth (e.g. Bjornsson et al. 2001; Peck et al. 2003). The effects of temperature have been studied in all the life history stages of Atlantic cod (Brander 1997). Atlantic cod juveniles have a larger thermal window width and tolerate a wider range of temperatures than adults and larvae (Lough 2004; Portner & Farrell 2008). Previous studies on juvenile cod have shown that swimming speed and growth rate increase with increased temperature (e.g. Bjornsson et al. 2001; Peck et al. 2006). However, compared to our understanding of the physiological responses to temperature change, our knowledge about the effects of temperature on the behavior of juvenile cod is limited.

Atlantic cod juveniles express a variety of territorial aggressive interactions (e.g. biting, chasing) and are likely organized in a dominance based hierarchy (Brawn 1961; Tupper & Boutilier 1995b, a; Hoglund et al. 2005). However, social interactions between juvenile cod individuals are not always so direct and are dependent on several ecological factors. Group behaviors such as aggregating and territorial defence are related to predation risk, habitat complexity and density of conspecifics (Gotceitas & Brown 1993; Tupper & Boutilier 1995b, a; Anderson et al. 2007). Juvenile cod can effectively reduce predation risk by reducing activity while sheltering or aggregating (Gotceitas et al. 1995; Laurel & Brown 2006). Moreover, when juveniles detect a potential predator, they may reduce the probability of being detected by ceasing all activity (i.e. "freeze response") (Smith 1997; Laurel & Brown 2006), or they attempt an escape when attacked with a high energy burst of swimming response (i.e. "fast start response") (Meager et al. 2006).

In the present study we examine the effect of temperature on social and feeding behavior in Atlantic cod juveniles. As juveniles must meet higher metabolic rates and energy demands with increasing temperature we also expect that activity, feeding attempts and willingness to forage in a risky environment will increase with temperature. In order to survive in a changing environment, organisms must be able to adapt to different conditions. Using their behavioral flexibility, individuals can overcome fitness trade-offs brought on by environmental change. We hypothesized that due to their lower energetic demands juveniles held at a lower temperature will have less feeding motivation but will also be more willing to respond to risk by adjusting their group behavior, i.e. by increased group cohesion, being less aggressive and spending more time sheltering. To test our hypothesis we examined space use, aggregation and feeding in three experimental environments, 1) control (no predator, no food), 2) feeding (no predator, with food) and 3) added risk (with food, following dip net chase) at the low and high ends of juvenile cod temperature tolerance limit.

Methods

Experimental animals

Juvenile Atlantic cod (*Gadus Morhua*), age 0+ were obtained from a local land based juvenile cod farm (Gunnvör hf). The fish were hatched from wild parents in September 2009. The cod juveniles were reared in an industrial fish farming environment, the rearing conditions were 8-10 kg/m3 with automatic feeding every hour and held under a 12:12 h light-dark regime. Fish were transported to the laboratory where they were held under a 12:12 h light-dark regime in two 400l tanks. Each tank was supplied with seawater (salinity of 30-35ppt). The tanks were cleaned every second day and maintained at a temperature of ≈9°C. Cod were fed a diet of dry commercial pellets (2.5-3 mm). For the purpose of our study we used 120 age 0+ juvenile cod (TL= 12.47±0.18).

Experimental setting

We used a temperature controlled aquarium system of 2 tanks (240x40x30cml), each divided into three compartments (80x40x30cm) by opaque plexiglass barriers. Our experiments were conducted under a 12:12 h light-dark regime and in water with a salinity of 30-35ppt. The flow rate of the water was 0.1 l sec^{-1}. The seawater flowed through a filtration sump tank and the system was given a partial water change and cleaned every second day. The experiments took place between February and April 2010.

The basic design of the experiment was first to monitor the behavior (aggregation; swimming activity; aggressiveness) of age 0+ juveniles at two different temperatures (3ºC-13ºC). Subsequently, we monitored their response and behavior

(aggregation; swimming activity; aggressiveness; feeding behavior) in the presence of food after starvation for 5 days and lastly we monitored their behavior (aggregation; swimming activity; aggressiveness; feeding behavior) following exposure to a stress stimulus (added risk) (Fig.1).

For each trial we randomly selected 3 fish that formed the test group. Each test group was placed in one of the three experimental aquarium compartments (80x40x30cml) each of which contained 4 sea stones (average size of 15- 20 cm) under which fish could shelter. Water temperature was maintained at an average of 3ºC for the first experiment, and an average of 13ºC for the second. We tested 20 groups of 3 juveniles in each temperature. Each fish was tested only once. Each trial lasted for 13 days and observations of behaviors were performed on days 7 and 13.

Each test group of fish was placed into the aquarium and acclimatized to the temperature for one week prior the experimental observations while fed to satiation. After acclimation we observed three behavioral traits: swimming activity, aggregation and aggressiveness for 20 min. After the first period of observations fish were left without food for 5 days to induce complete or partial stomach evacuation (Daan 1973; Steingrund & Ferno 1997) thus increasing feeding motivation (e.g. Meager & Batty 2007). We then introduced food pellets attached to a string (the fish could bite the food but were not able to consume it) into the center of the experimental tank and recorded fish behavior (swimming activity, aggregation, aggressiveness, feeding behavior) for 20 min. Immediately following that, we performed a 20 sec chase with a dip-net (6x6 cm green hand-net) to create a stressful experience. This was used to try and mimic a chase by a predator. Recovery from the stressor was monitored by observing behavior (swimming activity, aggregation, aggressiveness, feeding behavior; boldness) over 20 min. Boldness and risk taking was estimated as the

latency for the fish to recover from the simulated predation attempt and start their feeding attempts.

Digital video recordings were obtained by a remotely operated camera mounted 55cm directly above each compartment. Aggressive acts were defined as: (1) Approach: a fish approaches another with the mouth open and operculum flared (2) Nip/bite: a fish nips or bites another fish; and (3) Chase: an approach elicits a flight response, and the attacking fish follows the escaping fish for a distance of more than three body lengths (Hoglund et al. 2005). All aggressive interactions were counted and the sum used as one variable.

Video and data analysis

Swimming activity was measured using a free public domain software, ImageJ 1.42q (Rasband 2009) available at (http://rsb.info.nih.gov/ij/index.html). With MTrackJ (Java program for manual object tracking) plug-in v.1.3.0 (Meijering 2008) available at (http://www.imagescience.org/meijering/software/mtrackj/). We used ImageJ after calibrating it to provide data in meters. The MTrackJ plug-in was used to manually get the calibrated coordinates of each fish and the calculated distance traveled by the target fish between every frame (2sec). Method described by Myrick (2009). For the purpose of our study we took snapshots frame every 2sec of a 10min video recording in both temperature experiments and treatments. This means that we obtained 301 coordinates per fish per combination of temperature and treatment. As a measure of swimming activity we summed the 300 horizontal vectors of swimming distance (m) for each fish according to the formula

$$\sum_{i=1}^{301} \sqrt{[(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2]}$$

, were x_i and y_i are the x and y-coordinates for the ith frame.

Aggregation distances were measured using ImageJ. We used ImageJ after calibrating it to provide data in cm. We took a snapshot every 2.5 min thus obtaining a total of 9 images for each of our 20min video recordings. Distances between individual juvenile cod were measured by using a point between the eyes on the head. A total of 27 aggregation distances per test group were obtained.

For each test group we summed all aggressive acts, defined as approach, nip/bite and chase for each of the 20-min video recording, i.e. for all three treatments. Similarly, the sum of feeding attempts was recorded for the two feeding treatments i.e. the feeding and added risk treatments.

In order to examine both swimming activity and shoaling we used a linear mixed effects model (fixed and random) repeated measures ANOVA. Temperature (3ºC or 13ºC) and treatment (control, feeding or added risk) formed the fixed factors. The random effect factor, accounted for repeated measurements of group of fish (trials) within different temperatures and treatments. Further analysis was performed when significant interactions were found. We also used 2-way ANOVA to analyse the variation in aggressiveness and feeding attempts with respect to temperature levels and treatments and one-way ANOVA if significant interactions were found. One-way ANOVA was used also to analyse variations in the time needed by each group of fish to recover from hand-net chase and attempt to eat in both temperatures, using the total recovery time and total number of feeding attempts in each trial and treatment as variables, respectively. Aggression data, feeding attempts and time to recover were $\log(x+1)$ transformed prior to analyses. Using a 10min duration video from our trials we took fish coordinates in each 2sec frame and using the ImageJ and MTrackJ plug-in we estimated their utilization distribution (UD), by estimating the kernel home-

range (kernelUD) using library 'adehabitat' v.1.8.3 (Calenge 2009) in R statistical software. Using this model, we defined the home range as the minimum area in which an animal has some specified probability of being located. We performed all our statistical analysis using R statistical program (version 2.9.2 The R Foundation for Statistical Computing, 2009).

Results

Activity

Age 0+ cod swimming activity changed as a function of temperature ($F_{1,335}$= 68.27, p<0.001) and treatment ($F_{2,335}$=9.94, p<0.001) with a significant interaction between them ($F_{2,335}$= 5.32, p=0.0053) (Table 1). Further analyses showed that fish held at the temperature of 13ºC were more active than the fish held at 3ºC in all treatments (control; $F_{1,99}$=39.57, p<0.001; feeding; $F_{1,99}$=10.09, p=0.002; added risk; $F_{1,99}$=17.13, p<0.001) (Fig.2). Fish significantly changed their activity between the treatments at both experimental temperatures (for 13ºC, $F_{2,158}$=10.55, p<0.001; for 3ºC, $F_{2,158}$=4.1, p=0.01). Juveniles held at the higher temperature significantly changed their activity between control and feeding treatment ($F_{1,99}$=8.04, p=0.0055) and between control and added risk treatment ($F_{1,99}$=16.57, p<0.001). Juveniles held at 3ºC significantly changed their activity only between feeding and added risk treatment ($F_{1,99}$=8.93, p=0.0035) (Table 2).

Aggregation

Our analysis showed that age 0+ juvenile aggregations changed significantly in response to temperature ($F_{1,3215}$= 14.31, p<0.001) but not in response to the experimental treatment ($F_{2,3215}$= 1.19, p=0.30). The interaction between temperature and treatment was not significant ($F_{2,3215}$= 2.79, p=0.06) (Table 3). Further analysis was applied to analyze the aggregation distances in each temperature and in summary fish held at the 3 ºC temperature changed their aggregations between the treatments ($F_{1,1598}$=3.87, p=0.02) in contrast to fish from 13ºC that did not change their aggregations between the treatments ($F_{1,1598}$=0.18, p=0.83). Fish held at 3ºC

aggregated more closely in the feeding ($F_{1,1059}=5.89$, p=0.01) and added risk ($F_{1,1059}=14.65$, p<0.001) treatments (Fig.3). Fish held at both temperatures kept similar aggregation distances in the control treatment ($F_{1,1059}=0.16$, p=0.68). Juveniles held at 3ºC significantly changed their aggregation distances only between the control and added risk treatment ($F_{1,1059}=7.43$, p=0.0065) (Table 4).

Feeding attempts and boldness

There were highly significant differences in feeding attempts between temperature regimes (2-way ANOVA, $F_{1,76}=17.68$, p<0.001) and the two feeding treatments (2-way ANOVA, $F_{1,76}=13.22$, p<0.001) (Table 5). Age 0+ juveniles held at 13ºC had more feeding attempts than juveniles in 3ºC in both treatments (ANOVA, feeding; $F_{1,38}=9.06$, p=0.004; added risk; $F_{1,38}=8.63$, p=0.005) (Fig.4). The number of feeding attempts in both 3ºC (ANOVA, $F_{1,38}=4.13$, p=0.04) and 13ºC (ANOVA, $F_{1,38}=17.94$, p<0.001) temperature were significantly lower after the predator attack simulation (Fig.4). Fish from the 13ºC were faster at recovering and start their feeding attempts earlier compared to fish held at the 3ºC (ANOVA, $F_{1,34}=6.82$, p=0.01) (Table 6). In four (20%) of the trials, fish held at 3ºC did not recover from the simulation of the predator attack and did not start their feeding attempts within the 20min trials.

Aggression

Aggressiveness of age 0+ cod changed in response to temperature (2-way ANOVA, $F_{1,114}=34.27$, p<0.001) but not in response to treatment (2-way ANOVA, $F_{2,114}=0.56$, p=0.56). The interaction between temperature and treatment was not significant (2-way ANOVA, $F_{2,114}=1.29$, p=0.27) (Table 7). Therefore, we performed one-way ANOVA to test temperature effect on aggression in each treatment and our results

showed significant differences in the feeding and added risk treatment. Juvenile fish from the 13ºC temperature were more aggressive than the fish kept in 3ºC temperature when food was placed in the tank and after the predator attack simulation (ANOVA, control, $F_{1,38}=3.24$, p=0.07; feeding, $F_{1,38}=15.57$, p<0.001; added risk, $F_{1,38}=24.9$, p<0.001) (Fig.5).

Fish distribution

Using the home range analysis we estimated the density probabilities of the age 0+ juveniles inside the experimental compartment. Juveniles in both 13ºC and 3ºC temperatures preferred the corners with the shelter places in all treatments (control, feeding, added risk). Juveniles held at the temperature of 13ºC were more active with the probability contours covering more area inside the experimental compartment in contrast to juveniles held at 3ºC. Moreover, fish at 13ºC had higher probabilities to be found closer to the feeding site (centre of the tank) before and after the simulation of the predator attack (Fig.6.7).

Discussion

Temperature is one of the most important environmental factors to affect physiological and biochemical processes in fish (Brett 1979). Every response and process of fish, including reproduction, distribution, survival and feeding activity proceeds within the thermal range of each fish species. As hypothesized, we found differences in the swimming activity of Atlantic cod juveniles at 3°C and 13°C. Our results are in line with previous studies suggesting that temperature affects several aspects of juvenile cod swimming performance (e.g. Schurmann & Steffensen 1994; Castonguay & Cyr 1998; Winger et al. 2000). In the current study, age 0+ juveniles held at the higher water temperature had increased activity in all the experimental treatments used, showing that water temperature affected both the total distance moved and the total area covered (Fig. 2,6,7).

Feeding motivation is determined by an individual's metabolic demand, and as rising temperature (within the species´ temperature scope) increases metabolism it also influences hunger, feeding activity and feeding intensity (e.g. Jobling 1988; Stoner et al. 2006). Our results showed that fish held at the temperature of 13ºC spent significantly more time at the feeding site with more feeding attempts and thus higher feeding motivation compared to juvenile cod held at the temperature of 3ºC (Fig.4, 6, 7). Moreover, juvenile cod at the higher temperature commenced feeding 3 times faster after the simulation of the predator attack and were more exposed to the non shelter area at the center of the tank where the food was placed in order to overcome their higher energetic demands (Table 6). In a natural setting this could make juveniles at higher temperatures more vulnerable to predation.

Under the risk of predation, fish express several antipredator responses. Freeze (i.e. fish remained motionless) and escape (i.e. fish swam away from the fright stimuli with a fast response) are two of the main behavioral responses (e.g. (Laurel & Brown 2006; Domenici et al. 2007) used by the juveniles in our experiments. Juvenile fish held at both temperatures responded similarly when they were chased with the hand-net. However, fish held at the lower temperature were less active and spent more time at the shelter sites to recover after the predator attack simulation.

Aggregation and sheltering are two of the main strategies used by prey fish mostly to avoid detection and predation risk (e.g. Magurran 1990; Gotceitas et al. 1995). Animals adapt to their environment by adjusting behavioral strategies in response to their life experiences. Juvenile cod use both antipredator tactics; at the pelagic life stage aggregating is their primary strategy for predator avoidance but following their benthic settlement juvenile's become territorial making sheltering more likely (Gotceitas et al. 1995; Laurel & Brown 2006). In our study age 0+ juveniles used a sheltering strategy, presumably to minimize a perceived risk after the stress response. Studies have shown that guppies respond to increased predation risk by schooling. This response was found to be more vigorous at higher temperature in the presence of a predator (Weetman et al. 1998, 1999). In our study complex antipredator behavioral responses were not documented mainly because juveniles were equally exposed to the simulated predator attack at both temperatures. Aggregation distances in the control treatments did not differ between the temperatures with juveniles preferring the shelter areas in both cases. However, juveniles held at the temperature of 3ºC were more aggregated in the added risk treatment with most of them remaining closer to the corners of the experimental aquarium using the sea stones for shelter (Fig.3). Juvenile's inter-individual distances

did not change between the treatments, at the higher temperature. The increased tendency towards foraging rather than aggregating shown by the juveniles held at the higher temperature, after a simulated predator attack, suggests that the response is mainly due to their metabolic state. Moreover, lower aggressiveness can also explain why juvenile's sheltered and aggregated more at the lower temperature. In other words, because of higher metabolic demands and the direct influence on competition and aggressive interactions, juveniles held at 13°C have limited ability to adjust to other environmental changes including predation risk.

Atlantic cod juveniles have been shown to engage in aggressive territorial behaviors (e.g. biting, chasing) and be organized in a dominance based hierarchy (Brawn 1961). In our study juveniles held at the temperature of 13ºC were significantly more aggressive than juveniles in the 3ºC when food was introduced to the centre of the experimental tank, both before and after the simulation of a predator attack. This was not seen in the absence of food (Fig.5). Further studies are needed, for further understanding the effect of temperature and feeding on aggressiveness in Atlantic cod juveniles. However, both higher levels of aggression and activity are likely to affect juvenile aggregation distances and may be the main reason for less aggregation at the higher temperature. Higher activity levels have been documented to increase feeding and growth rates but reduce survivorship in many animal taxa including fish, the latter effect being due to longer exposure to and thus higher encounter rates with predators (Werner & Anholt 1993; Biro et al. 2003; Biro et al. 2007). In our study, home range analysis showed higher probabilities for the fish held at the temperature of 13ºC to be found closer to the feeding area compared with fish held at 3ºC after the simulation of a predator attack (Fig.6,7). This is suggestive as to

how predator-prey dynamics in the wild could change with increasing temperature e.g. by increased predator - prey encounter rates leading to higher prey fish mortality.

In conclusion, the results of our study suggest that several aspects of Atlantic cod juvenile social behavior (e.g. swimming activity; aggregation; boldness; aggressiveness) and feeding related behaviors are significantly affected by water temperature. Fish held at higher temperatures are generally more active but alter their behavior less when faced with increased risk. We propose that either because of consequential increase in activity at higher temperature or because of their high metabolic demands juvenile cod held at 13°C have limited scope to reduce their feeding activity and are forced to remain active when faced with risk. Our results are in line with previous studies suggesting that under the scenario of increased sea temperatures, several aspects of juvenile cod behavior, related to predation vulnerability (e.g. activity, boldness) are likely to be disrupted leading to changes in fish populations.

Acknowledgements

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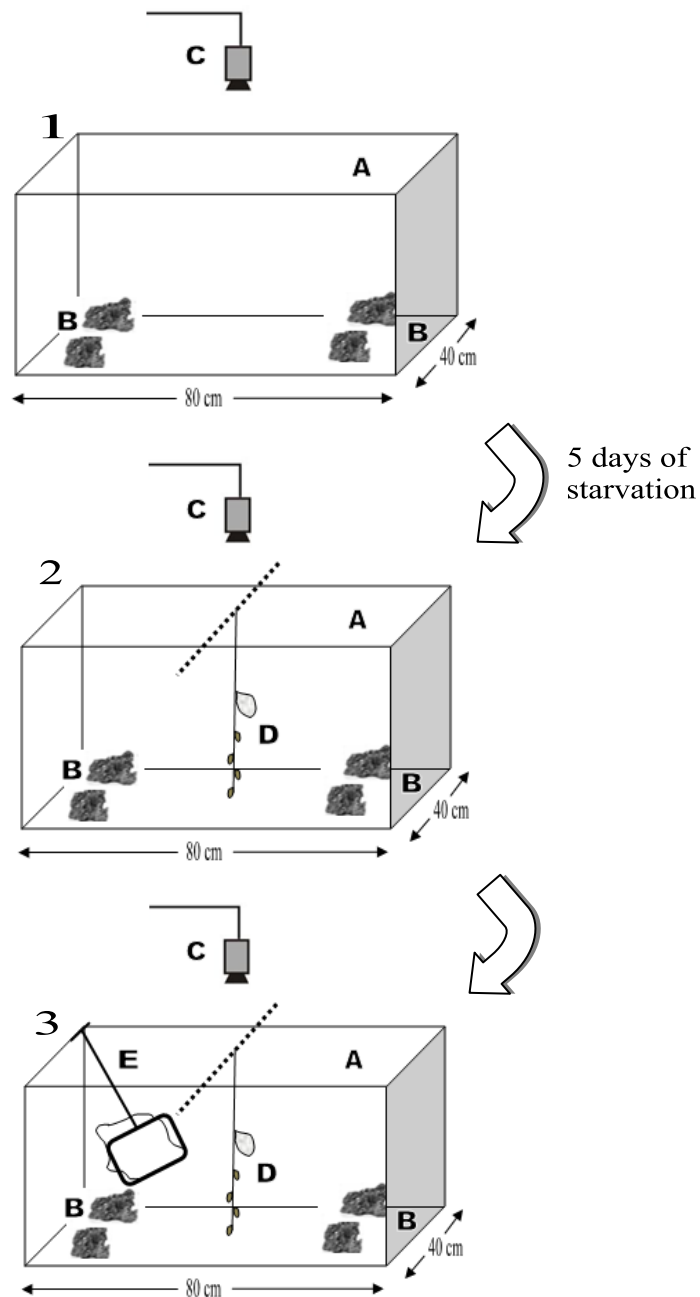
Figures

Figure 1. Experimental set-up and procedure. Experimental compartment used to analyze (1) juvenile behavior (control treatment), (2) same juvenile group feeding behavior after 5 days of starvation (feeding treatment) and (3) immediately after feeding behavioral observations the use of hand-net to observe stress response (added risk treatment). (A) Experimental compartment (80x40x30 cm). (B) Shelter sides. (C) Video camera. (D) Food pellets attached to a string on non-shelter side. (E) Hand-net used to create stressful experience and mimic predator attack for 20sec.
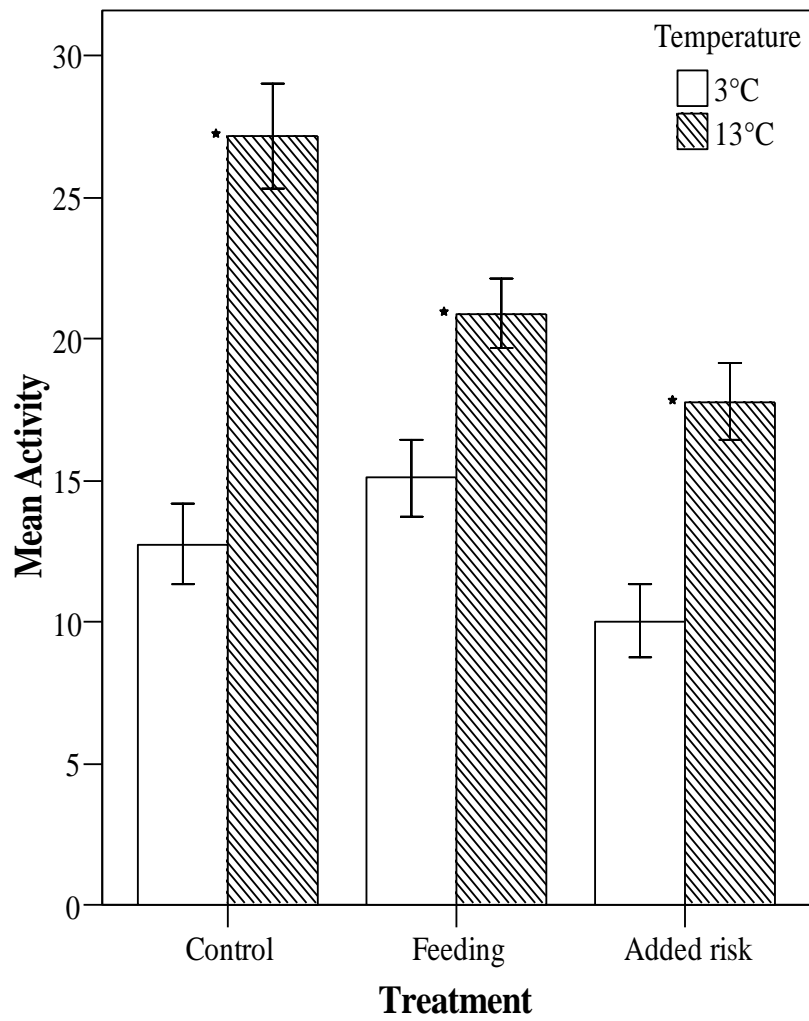
Figure 2. *Mean (±1 SE) activity (distance moved in meters) in all treatments. Control (no predator, no food), feeding (no predator, with food) and added risk (with food, following dip net chase), over a period of 10 min in 3℃ and 13℃ temperatures. * indicates a significant difference between the two temperatures.*
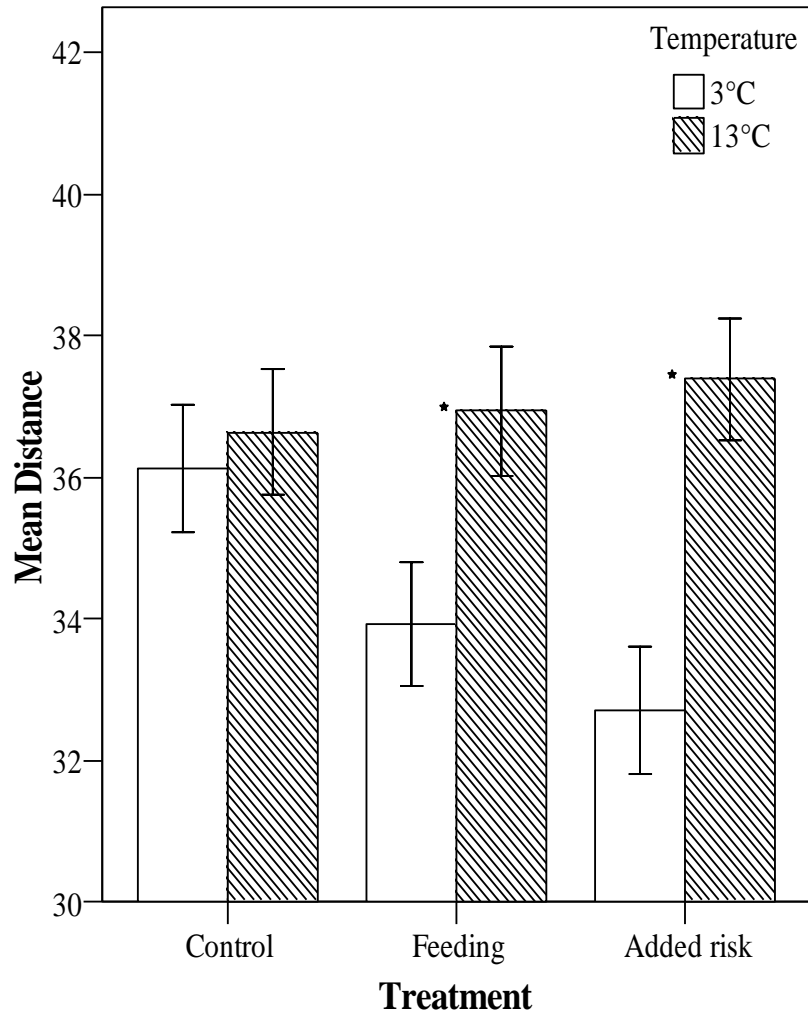
Figure 3. *Mean (±1 SE) distances (cm) among juveniles in all treatments. Control (no predator, no food), feeding (no predator, with food) and added risk (with food, following dip net chase) over a period of 20 min in 3ºC and 13ºC temperatures. * indicates a significant difference between the two temperatures.*
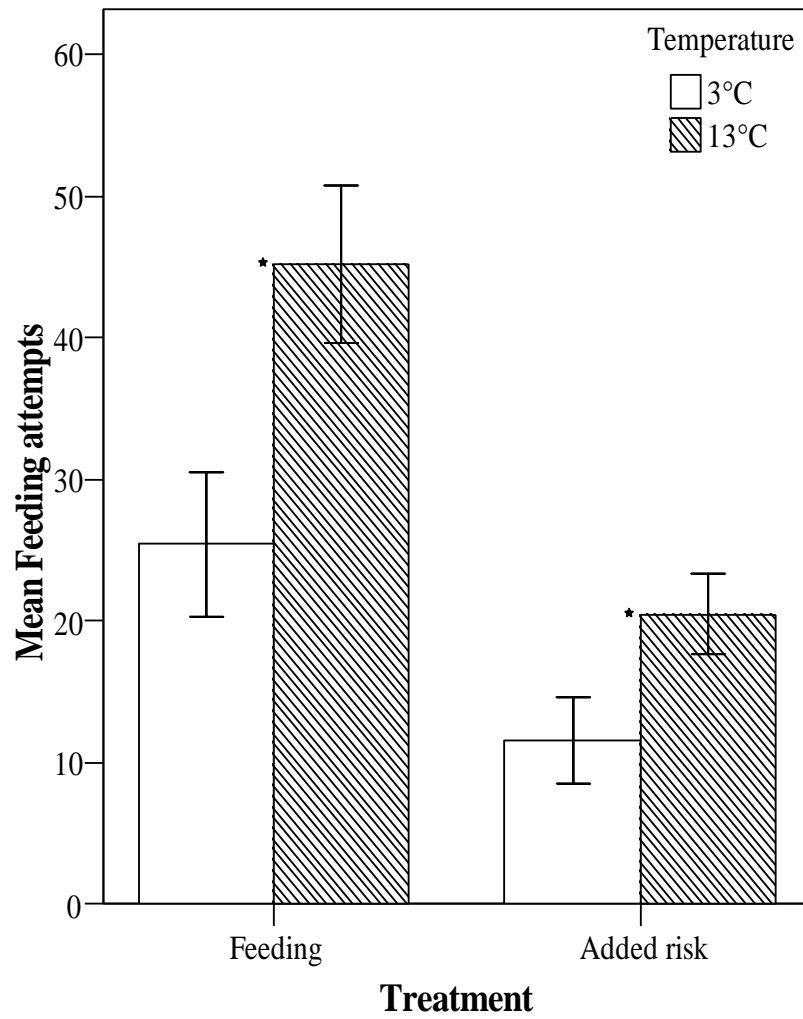
Figure 4. *Mean (±1 SE) number of feeding attempts before and after the simulation of a predator attack in 3℃ and 13℃. * indicates a significant difference between the two temperatures.*
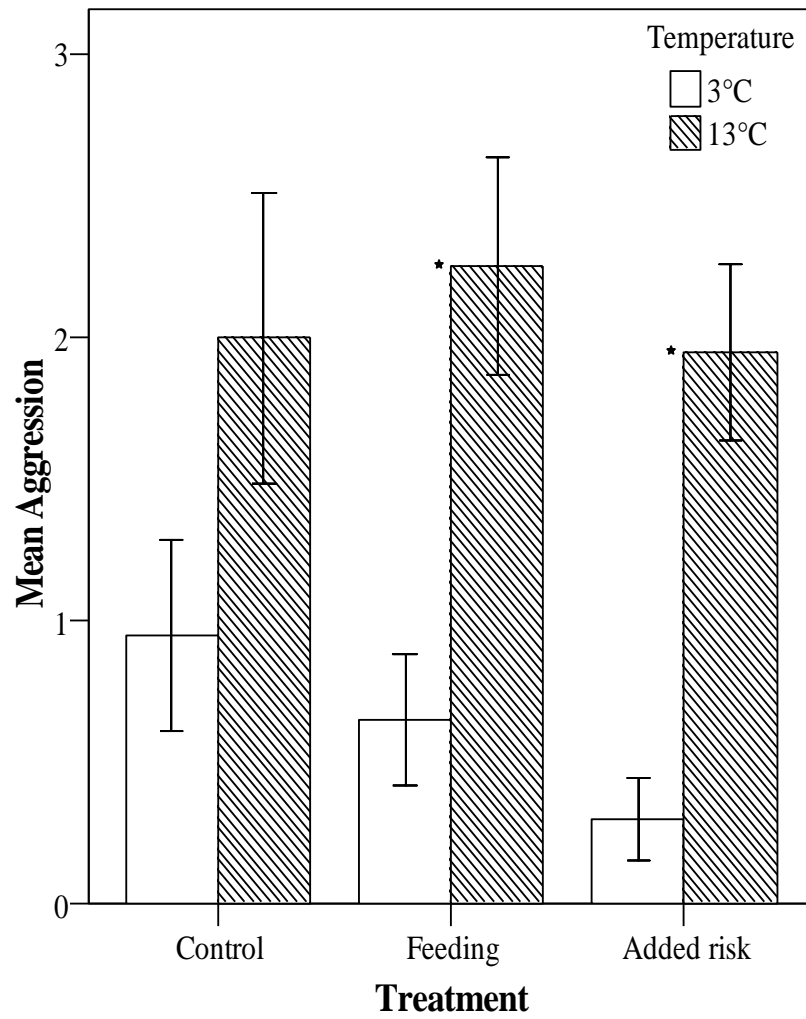
Figure 5. Mean (±1 SE) number of aggressive acts of age 0 juveniles in 3℃ and 13℃ in all treatments (control; feeding; added risk). * indicates a significant difference between the two temperatures.

Figure 6. *The graphics show the kernel home range utilization distribution (probabilities) using the ad hoc calculation for smoothing parameter for the age 0+ juveniles held at 3°C temperature. The darker regions in the plot correspond to higher probabilities. (A) Control, (B) feeding, (C) added risk treatment.*
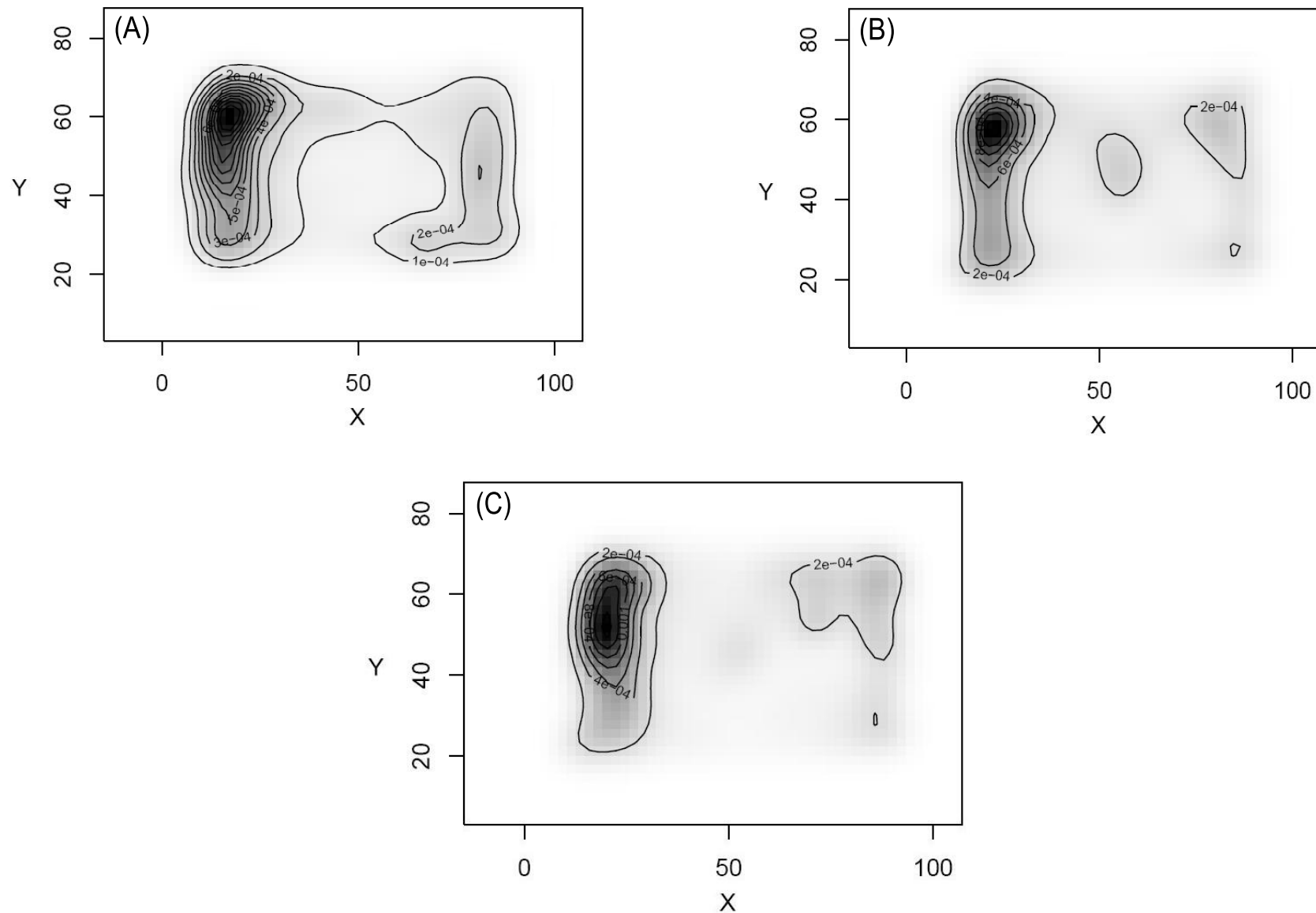
Figure 7. *The graphics show the kernel home range utilization distribution (probabilities) using the ad hoc calculation for smoothing parameter for the age 0+ juveniles held at 13°C temperature. The darker regions in the plot correspond to higher probabilities. A) Control, (B) feeding, (C) added risk treatment.*
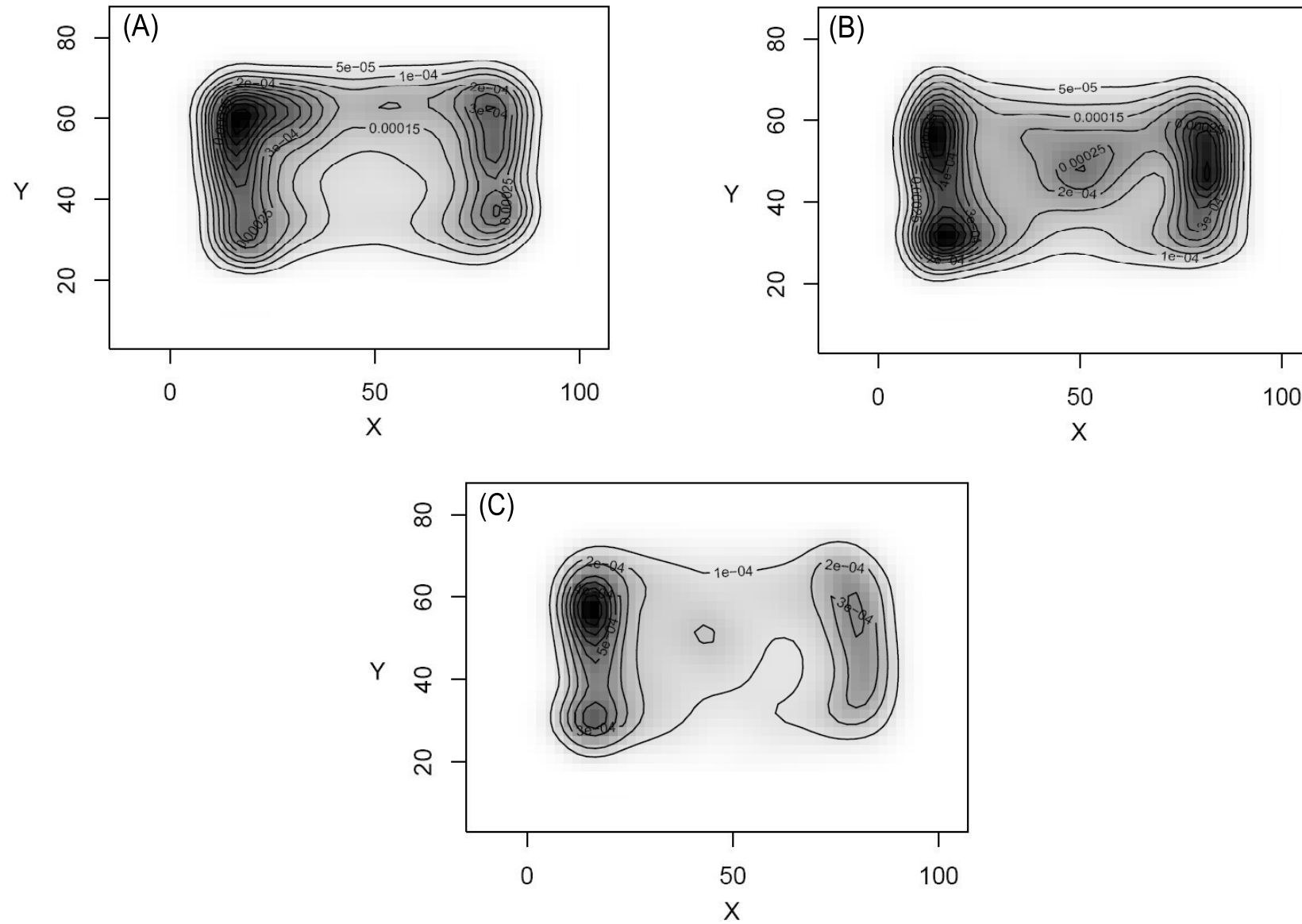
Tables

Table 1. *Analysis of variance showing effects of temperature and treatment on juvenile swimming activity (m).*

	Source	numDF	denDF	F-value	*p*-value
F	(Intercept)	1	335	380.56	< .001
	Temperature	1	335	68.27	<.001***
	Treatments	2	335	9.94	0.001***
	Temperature:Treatments	2	335	5.32	0.0053**

***Table 2**. Analysis of variance for juvenile swimming activity compared between treatments in each of the tested temperatures.*

	Source	numDF	denDF	F-value	p-value
3°C	Control v. Feeding	1	99	1.56	0.21
	Control v. Added risk	1	99	2.26	0.13
	Feeding v. Added risk	1	99	8.93	0.0035**
13°C	Control v. Feeding	1	99	8.04	0.0055**
	Control v. Added risk	1	99	16.57	< 0.001***
	Feeding v. Added risk	1	99	2.99	0.08

Table 3. *Results from variance showing the effects of temperature and treatment on juvenile aggregation.*

	Source	numDF	denDF	F-value	*p*-value
F	(Intercept)	1	3215	3632.96	$< .001$
	Temperature	1	3215	14.31	<0.001***
	Treatments	2	3215	1.19	0.30
	Temperature:Treatments	2	3215	2.79	0.06

Table 4. Analysis of variance or juvenile aggregations compared between treatments in each of the tested temperatures.

	Source	numDF	denDF	F-value	*p*-value
3ºC	Control v. Feeding	1	1051	3.12	0.077
	Control v. Added risk	1	1051	7.43	0.0065**
	Feeding v. Added risk	1	1051	1.00	0.31
13ºC	Control v. Feeding	1	1051	0.06	0.80
	Control v. Added risk	1	1051	0.37	0.54
	Feeding v. Added risk	1	1051	0.13	0.71

Table 5. Results from ANOVA showing effects of temperature and treatment on juvenile feeding attempts.

	Source	d.f.	SS	MS	F	P
F	Temperature	1	3.31	3.31	17.68	< 0.001***
	Treatment	1	2.47	2.47	13.22	< 0.001***
	Temperature:Treatment	1	0.0004	0.0004	0.002	0.96
	Residuals	76	14.23	0.18		

Table 6. *Descriptive statistics for the timing of the first feeding attempt after the predator simulation at the two experimental temperatures.*

Temperature (ºC)	N	First feeding attempt after predator attack simulation (min) ± Std Error
3ºC	16	6.04 ± 1.31
13ºC	20	2.8 ± 0.38

Table 7. *Results from ANOVA showing effects of temperature and treatment on juvenile cod aggressiveness.*

	Source	d.f.	SS	MS	*F*	*P*
F	Temperature	1	2.1015	2.10145	34.2703	< 0.001***
	Treatment	2	0.0698	0.03492	0.5695	0.56
	Temperature:Treatment	2	0.1585	0.07924	1.2923	0.27
	Residuals	114	6.9905	0.06132		