Increased mortality of growth-enhanced transgenic common carp (Cyprinus carpio L.) under short-term predation risk

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Summary

There is strong evidence that genetic capacity for growth evolves toward an optimum rather than an absolute maximum. This implies that fast growth has a cost and that trade-offs occur between growth and other life-history traits. In this study, we conducted a series of predation trials with a paired-contrast design to test the differences in anti-predator ability between growth-enhanced transgenic and non-transgenic common carp (Cyprinus carpio L.). We showed that young transgenic fish suffered higher predation mortality than control carp in both small-bodied and large-bodied size-matched trials, and thus possessed lower anti-predator ability. Our results suggest that a trade-off exists between growth rate and survival such that rapid growth entails a cost in terms of mortality.

Introduction

In the early 1980s, the gene construct MTGH human growth hormone (GH) gene driven by mouse metallothionein-I (MT-I) promoter was microinjected into the fertilized eggs of goldfish (Carassius auratus L.), producing a first batch of fast-growing transgenic fish (Zhu et al., 1985). Under the consideration of bio-safety and bio-ethics, MTGH construct is not encouraged for use in the breeding of fish. Thus the recombinant transgene construct, pCAgcGH, made up of all fish (Cyprinidae) genomic elements (e.g. the grass carp GH structural gene spliced under a common carp β-actin gene promoter) was transferred into common carp (Cyprinus carpio L.), which led to the birth of ‘all fish’ GH-transgenic common carp (Zhu et al., 1992). In order to differentiate the gene construct MTGH, the recombinant construct pCAgcGH is often called the ‘all fish’ GH gene. The GH-transgenic common carp grew 1.4–1.9 times that of non-transgenic counterparts under hatchery conditions (Wang et al., 2001; Li et al., 2007). GH-transgenic fish are of great commercial interest in aquaculture, but also raise concerns with regard to the potential impacts of escaped or released GH-transgenic fish on aquatic ecosystems (Maclean and Laight, 2000). To predict these risks, it is crucial to obtain empirical data on the relative fitness of GH-transgenic and non-transgenic fish under simulated natural conditions.

Rapid growth is often perceived as being beneficial, especially in juvenile stages (Sogard, 1997). In many fish species, fast growth should increase fitness since larger fish at a given age can achieve higher dominance status, reduce their susceptibility to predators (Juanes et al., 2002) and increase their reproductive potential (Fleming, 1996). Evidence has accumulated indicating a trade-off between benefits and costs associated with rapid growth (Arendt, 1997; Mangel and Stamps, 2001; Morgan and Metcalfe, 2001; Munch and Conover, 2003; Biro et al., 2005), suggesting that limitations exist with the concept that faster growth is always better. Recent studies with fast-growing fish have shown decreased escape performance (Lankford et al., 2001; Munch and Conover, 2003), less vigilance and more willingness to predation risk when foraging (Johnsson et al., 1996; Abrahams and Pratt, 2000), and greater predation mortality (Biro et al., 2004) with increases in growth rate. Similar results were found in GH-transgenic fish relative to their non-transgenic counterparts (Farrell et al., 1997; Abrahams and Sutterlin, 1999; Sundström et al., 2004). These findings indicate that the fitness advantage of rapid growth may be balanced by a variety of associated costs.

Predation is an important factor structuring fish populations in nature (Whoriskey and Fitzgerald, 1985; Persson, 1991). By killing prey, predators also can control prey populations, drive some types of prey to extinction, and alter the relative and absolute abundances of prey (Sih et al., 1985). Small fish are more vulnerable to predators, and few individuals will survive to maturity in the presence of various predators (Sogard, 1997). For juvenile fishes, an innate ability to recognize and avoid natural predators can have fitness advantages (Sogard, 1997). It is therefore essential to obtain data on the survival fitness of GH-transgenic and non-transgenic fish exposed to predators during their earlier life-history stages.

In this study, we used survival after short-term risk of predation to compare the anti-predator ability of GH-transgenic and non-transgenic common carp at the juvenile stage. Such information would be useful for further examination of the trade-off between rapid growth and mortality. Also, this would be essential for evaluating potential impacts that transgenic fish may have on aquatic ecosystems.

Materials and methods

Source of fish

P0 GH-transgenic common carp were initially produced by micro-injection of pCAgcGH into fertilized eggs of common carp (Yellow River variety). Gene construct pCAgcGH was a recombinant construct of the grass carp Ctenopharyngodon idella (Valenciennes) growth hormone gene (gGH), whose expression is driven by the β-actin gene promoter of common carp (pCA). The transgene was integrated, expressed, and

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inherited in the subsequent F1 and F2 generations in a Mendelian fashion. Experimental GH-transgenic fish (T) and their non-transgenic all-siblings (NT) used in this study were F1 generation progeny, produced by crossing a wild type female with an F2 transgenic heterozygous male of a fast-growing transgenic germ line. Fertilized embryos from this cross were incubated on 14 April 2005, which theoretically yields 50% T and NT offspring each. Hatched first-feeding fry were reared in eight outdoor rectangular concrete tanks (each 10 m²) at different stocking rates. Fish first fed on plankton and then consumed commercial diets when over 1.8 cm TL. At 8 weeks of age they were transported to the laboratory aquaria for identification. The left or right ventral fin was partially clipped and used to identify the \( pC4gaGH \) transgene-positive fish by the PCR method following Wang et al. (2001). All experimental T (positive) and NT (negative) fish were selected from those individuals that completely regenerated the clipped fins after more than 4 weeks.

Mandarin fish, \( S. \) \( . \) \( c. \) \( a. \) \( r. \) \( a. \) (Basilewsky), was chosen as a predator in this study. It is a demersal large-sized piscivorous predator, widely distributed in many rivers and lakes of China. The fish have peculiar feeding habits and eat only live larvae of fish and shrimps when first feeding and refuse to consume dead prey or artificial diets during all life-history stages (Liang et al., 1998). Age 1+ mandarin fish in Liangzi Lake were captured with trap-nets and 15 individuals (mean mass ± SD: 482.3 ± 15.6 g) were selected for use in this study. Prior to the experiment, the mandarin fish were given at least 1 week to acclimate to confined concrete-tank conditions and fed with live crucian carp (\( C. \) \( a. \) \( r. \) \( a. \) \( t. \) \( u. \) \( r. \) \( a. \) \( t. \) \( u. \) \( s. \) \( ) \) juveniles once a day during acclimation.

**Experimental procedures**

The non-commercial research facility at the Fish Behavioral Ecology Laboratory of the Institute of Hydrobiology in China had multiple containment screen systems and was specially designed to prevent transgenic fish from escaping into nature.

A paired-contrast design tested differences in anti-predator ability of T and NT fish: Mixed schools containing an equal number of size-matched T and NT fish were exposed simultaneously to a predator. Two predation trials on T and NT fish were conducted from 18 August to 9 September and different trial durations were determined based upon the previous observations. The first trial (I) lasted 5 days, with a small-bodied size-matched population (\( T = 60, NT = 60 \) in one tank (see below). The second trial (II) lasted 3 days, with a large-bodied size-matched school (\( T = 30, NT = 30 \) in another tank. Prior to each trial, the left ventral fin was completely clipped for each T fish whereas the right ventral fin was clipped for each NT fish so that the two genotypes could be differentiated visually. To verify that the difference in predation mortality between T and NT fish was not derived from the fin-marking position (left vs right) and other non-treatment factors such as size, predator, and rearing facilities, an additional two predation trials (III and IV) were conducted using only NT fish separated into two groups and marked by an alternating ventral fin clip (left or right).

In all trials, experimental fish were reared in large, concrete tanks (3.5 m × 2.3 m × 1.5 m) to minimize confinement of prey and to permit them to express their natural flight response. A net-cover was put on each tank to keep aerial predators such as birds and cats away. Fifteen earthen flowerpots (three in each corner and three in the middle) were placed in each tank to provide refuge for prey. Experimental fish were maintained under ambient temperature (mean ± SD: 23.6 ± 2.6°C) and photoperiodic conditions, and fed a commercial diet twice (09.00 h and 14.00 h) daily. Tanks were supplied with flow-through filtered natural water drawn from Liangzi Lake, with pH 7.9 and dissolved oxygen > 6.9 mg/l. In each trial, three mandarin fish were chosen randomly as predators and placed into the experimental tank. During each trial, predation was the only source of mortality for both T and NT fish.

Prior to each trial, experimental fish were lightly anaesthetized with Eugenol, weighed (± g) and total length measured (± cm); the same procedure was carried out for the survivors at the end of each trial. Condition factor (CF) was calculated as:

\[
CF(\text{g cm}^{-3}) = 100 \times W/L^3
\]

Where \( W \) (g) is body mass and \( L \) (cm) is total length.

**Data analysis**

The differences between two mean values in total length, body mass and condition factor were examined by Students' \( t \)-test. The chi-square test was used to evaluate differences in mortality rate between two subpopulations in each predation trial. Differences were regarded as significant when \( P < 0.05 \). All statistical analyses were performed using the STATISTICA program by StatSoft, Inc. (1995).

**Results**

Data on initial total length, body mass and condition factor of experimental fish in four predation trials are shown in Table 1. In these morphological measures, there were no significant differences between two size-matched subpopulations at the start of each predation trial (Students' \( t \)-test, all \( P > 0.05 \); Table 1).

In trial I with small-bodied size-matched population, mortality rate of the T fish was 88.3%, significantly higher than that (61.7%) of the NT fish (\( x^2 = 11.38, P = 0.001, \) Fig. 1)). Similar results were found in trial II with the large-bodied size-matched population; mortality rate of the T fish was about two times that of the T fish (53.3% vs 26.7%, \( x^2 = 4.44, P = 0.035, \) Fig. 1).

In contrast to trial I, mortality rate of the trial III NT fish with the left ventral fin clipped was same as NT fish with their right ventral fin clipped, as occurred in trial IV contrasted to trial II (Fig. 1), which showed that the size-matched individuals of the same genotype possessed the same anti-predator ability. This also demonstrated that the fin-clip positions (i.e. left vs right) and other non-treatment factors (approximate size match, predator selection and experimental facilities, etc.) did not yield differences in predation mortality, and that differences in mortality between T and NT fish can be attributed to genotype in trials I and II.

**Discussion**

In the present study, replicated treatments in trials I and II were not designed because of the scarcity, difficulty in size match, and high cost of GH-transgenic common carp. We took advantage of a limited supply of the GH-transgenic fish. Our results demonstrate that GH-transgenic carp juveniles suffer increased predation mortality and possess lower
anti-predator ability. These findings are consistent with the study of Sundström et al. (2004), who showed that predation mortality of first-feeding fry of GH-transgenic coho salmon, Oncorhynchus kisutch (Walbaum), is much higher than that of non-transgenic control fry under near-natural conditions. Similar results were obtained in another study showing increased susceptibility to predators for GH-transgenic channel catfish, Ictalurus punctatus (Rafinesque), at an early life stage (Dunham et al., 1999).

In contrast to the present study, Tymchuk et al. (2005) found that GH-transgenic coho salmon at the parr stage did not suffer higher rates of mortality than non-transgenic counterparts and that they maintained their considerably enhanced growth when feeding in the presence of a predator. Johansson and colleagues also found that GH-implanted wild brown trout (Salmo trutta L.) grew faster than control trout in the wild without suffering increased mortality (Johnsson et al., 1999; Johnsson and Björnsson, 2001), although the effect of GH-treatment was stronger in the hatchery where food was more abundant (Johnsson et al., 2000). Those studies differ from the present study in that the experiments were conducted over a relatively long time period (over 3 weeks), in which fast-growing GH-implanted or GH-transgenic fish may be allowed to attain the expected benefits of large size. However, in those studies, there actually was an overall tendency for higher mortality in GH-transgenic or GH-implanted fish than in control fish. In addition, experiments on Atlantic salmon (Salmo salar L.) suggest that maintenance of energy reserves over winter is critical for fitness (Bull et al., 1996); previous work on brown trout indicated that GH-implanted fish increase growth rate at the cost of reduced energy reserves (Johnsson et al., 1999, 2000). Consequently, high GH expression in GH-transgenic or GH-implanted fish may eventually increase mortality by reducing energy reserves necessary for survival under harsh conditions.

Our results suggest that trade-offs exist between growth rate and fitness in the transgenic common carp and rapid growth entails a cost in terms of mortality. There are several possible explanations for these findings. In parallel experiments in earthen ponds where supplemental feed was applied, the same groups of transgenic common carp used in this study grew 40% to 90% faster than non-transgenic siblings (Li et al., 2007). To maintain increased rates of growth, fish must make trade-offs between foraging effort and risk of predation (Abrahams and Dill, 1989). Previous studies have indicated that GH-transgenic fish have a greater overall appetite and a much higher consumption rate (Abrahams and Sutterlin, 1999; Devlin et al., 1999; Fu et al., 2007; Duan et al., 2009) and thereby appear more active and risk-prone to forage (Abrahams and Sutterlin, 1999; Sundström et al., 2003) than

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**Table 1**

Data (mean ± SD) on initial total length (L), body mass (W) and condition factor (CF) of GH-transgenic (T) and non-transgenic (NT) common carp Cyprinus carpio in four predation trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Genotype</th>
<th>Ventral fin clipped</th>
<th>Number of fish</th>
<th>L (cm)</th>
<th>W (g)</th>
<th>CF (g cm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T</td>
<td>Left</td>
<td>60</td>
<td>6.63 ± 0.34</td>
<td>4.0 ± 0.6</td>
<td>1.365 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>Right</td>
<td>60</td>
<td>6.63 ± 0.23</td>
<td>4.0 ± 0.4</td>
<td>1.365 ± 0.003</td>
</tr>
<tr>
<td>II</td>
<td>T</td>
<td>Left</td>
<td>30</td>
<td>8.03 ± 0.32</td>
<td>7.8 ± 0.9</td>
<td>1.504 ± 0.129</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>Right</td>
<td>30</td>
<td>8.12 ± 0.23</td>
<td>7.2 ± 0.8</td>
<td>1.351 ± 0.085</td>
</tr>
<tr>
<td>III</td>
<td>NT</td>
<td>Left</td>
<td>60</td>
<td>6.41 ± 0.18</td>
<td>3.7 ± 0.4</td>
<td>1.386 ± 0.100</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>Right</td>
<td>60</td>
<td>6.37 ± 0.18</td>
<td>3.6 ± 0.5</td>
<td>1.374 ± 0.098</td>
</tr>
<tr>
<td>IV</td>
<td>NT</td>
<td>Left</td>
<td>30</td>
<td>8.36 ± 0.27</td>
<td>8.2 ± 1.1</td>
<td>1.397 ± 0.102</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>Right</td>
<td>30</td>
<td>8.39 ± 0.24</td>
<td>8.3 ± 1.0</td>
<td>1.399 ± 0.079</td>
</tr>
</tbody>
</table>

*Notes:* I, small-bodied size-matched population with T and NT subpopulations; II, large-bodied size-matched population with T and NT subpopulations; III, small-bodied size-matched population with only NT fish; and IV, large-bodied size-matched population with only NT fish. For all parameters there were no significant differences between T and NT fish examined by Student’s t-test at the level of P = 0.05.

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**Fig. 1.** Mortality rates of GH-transgenic (T) and non-transgenic (NT) common carp Cyprinus carpio at end of (a) trial I (5 days, small-bodied size-matched population) and trial II (3 days, large-bodied size-matched population); (b) trial III (5 days; small-bodied size-matched population) and trial IV (3 days; large-bodied size-matched population) with three predator mandarin fish. Black and open bars = left ventral fin clipped; gray bars = right ventral fin clipped. Asterisks = significant differences in mortality rate between the two genotypes in the same predation trials (Chi-square test, P < 0.05).
non-transgenic fish. Moreover, increased foraging activity level (Abrahams and Sutterlin, 1999) may reduce vigilance and/or increase the probability of encountering or being detected by predators (Werner and Anholt, 1993; Anholt and Werner, 1998). Finally, some studies have indicated that GH-transgenic common carp and salmon juveniles can be inferior swimmers, with significantly decreased critical swimming speeds compared with control fish of the same size (Farrell et al., 1997; Li et al., 2007), which may affect their escape performance. Similar results were also found in the study of Farrell et al. (1997), which showed that growth-enhanced GH-transgenic salmon juveniles had critical swimming speeds half those of size-matched control fish.

The present study indicated that GH-transgenic common carp at the juvenile stage showed lower survival under a short-term risk of predation. However, it is difficult to extrapolate our limited results into the natural environment because environmental complexity can greatly alter the phenotypic effects of the GH transgene (Sundström et al., 2007). Thus, we should use the results with caution when assessing ecological risk of GH-transgenic common carp. Future research is needed to understand key variables (Devlin et al., 2006) influencing survival fitness for risk assessment of transgenic fish because disadvantages in one attribute can be offset by advantages in another (Muir and Howard, 1999).

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