

The ones that got away:  
Resource overlap and competition between a non-native fish (*Oreochromis  
niloticus*) and Thai native species, and implications for ecological risk  
assessment

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## Abstract

Effects of non-native fish in tropical freshwater systems are commonly presumed but poorly understood. I present an approach to assess potential adverse changes in the native fish assemblage resulting from the presence of feral *Oreochromis niloticus*, which has an expansive naturalized range. This research is organized around an ecological risk model, consisting of a fault-tree of events. I present my findings for exposure events (site, habitat and dietary overlap) and effects events (interference competition and change in growth rates), which may lead to adverse changes in native assemblages in streams in the Eastern River system in Thailand.

My research addresses two assumptions regarding the ecological risk assessment of *O. niloticus* in Thailand, that native piscivores, *Channa* spp., limit population establishment and hence ecological risk, and an economically important native species, *Trichogaster trichopterus*, is declining due to competition with *O. niloticus*. I found that *Channa* spp., *T. trichopterus*, and *O. niloticus* were syntopic and had high dietary overlap. When present, *O. niloticus* were more highly abundant than other species; which suggests their successful establishment. Native fish assemblages at *O. niloticus* sites were not significantly different from those at un-invaded sites, and non-native species were the only strong indicators of sites with non-natives present. Therefore, *Channa* spp. did not limit establishment of *O. niloticus*, and *T. trichopterus* could be negatively affected via competition with juvenile *O. niloticus*.

I then tested whether *O. niloticus* adversely affect *T. trichopterus* through competition in laboratory experiments to determine the mechanism and outcome of competition between individuals and species in single and mixed species treatments at varying densities. Dominant fish in each tank grew more than subordinates, and *O. niloticus* were the dominant species in 67.7% of all mixed species tanks. The growth rate of *T. trichopterus* declined significantly in the presence of selectively bred *O. niloticus*, but the growth rate of *O. niloticus* was not noticeably affected by the presence of *T. trichopterus*. My study documents resource overlap, resource competition, and the effects of competition on growth. As such, it provides valuable insight into the ecology and conservation of native aquatic biodiversity in tropical freshwater systems.

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## Prologue

Non-native fishes are commonly thought to adversely change native fish assemblages, both through their ability to outcompete native species for shared resources and through direct predation on native species. The majority of research on the ecological effects of non-native fish introductions from aquaculture escapees and intentional introductions concern salmonids in well-studied temperate ecosystems (Pascual 2002). Comparatively little is known about the ecology and biology of both native and introduced fishes in the tropical ecosystems of Asia (Dudgeon 2003), where threats to native biodiversity are numerous, a diverse array of native and non-native species are cultured with little or no confinement, and fish biodiversity is high (Coates 2002).

Wild-caught fisheries are an important cultural, dietary, and economic resource in Thailand. *Oreochromis niloticus*, native to the Nile River basin in Africa, were introduced to Thailand after World War II to increase food production. Feral populations are now common and their effects on native species are widely speculated (De Silva et al. 2004). *Trichogaster trichopterus*, a native gourami, is valued commercially as an ornamental species and as a food fish. Both species build nests, defend territories, and provide parental care. Populations of *T. trichopterus* are thought to be in decline due to competition with feral *O. niloticus*; however, few empirical data are available to substantiate this and other claims regarding the effect(s) of *O. niloticus* on native species.

My dissertation research presents a step-by-step approach for assessing the potential for adverse ecological effects of a non-native fish using a fault tree (Figure 1). The fault tree traces a logic pathway from ecological hazard to ecological consequence using events, which are represented by boxes in the figure, that are necessary for a non-native fish species (the ecological stressor) to cause an adverse change in the native fish assemblage (the ecological response) (Burgman 2005). There are two components to the fault tree, exposure events and effects events. I present empirical findings from the field for exposure events (levels I, II, and III) in chapter 1, and empirical findings from the laboratory for effects events (levels IV and V) in chapter 2. Specifically, I present results on the establishment of the non-native species, and habitat and dietary overlap in syntopic populations in small streams in southeastern Thailand; and I document the mechanism of interference competition between the non-native species and a Thai native species and its outcome on the growth of both species. The levels (I – VI) of Figure 1 are parenthetically referenced throughout each chapter of the dissertation.

If non-native fish escape to an environment that is able to support their survival and there are native fish present in the ecosystem (level I) this forms the foundation for these species to interact. If level I is satisfied and there is establishment of the non-native (level II), via reproduction of non-native fish or their continuous immigration from fish farms, and the species share resources such as food and habitat (level III), the opportunity for competition exists (level IV). Competition is one of the most important mechanisms for individuals to affect one another. It can lead to direct or indirect effects

(level V) that may lead to an adverse change in the fish assemblage and a loss of native biodiversity (level VI). Risks to the assemblage include any adverse effects on native species or ecological processes that affect the assemblage (Devlin et al. 2007; Parker et al. 1999).

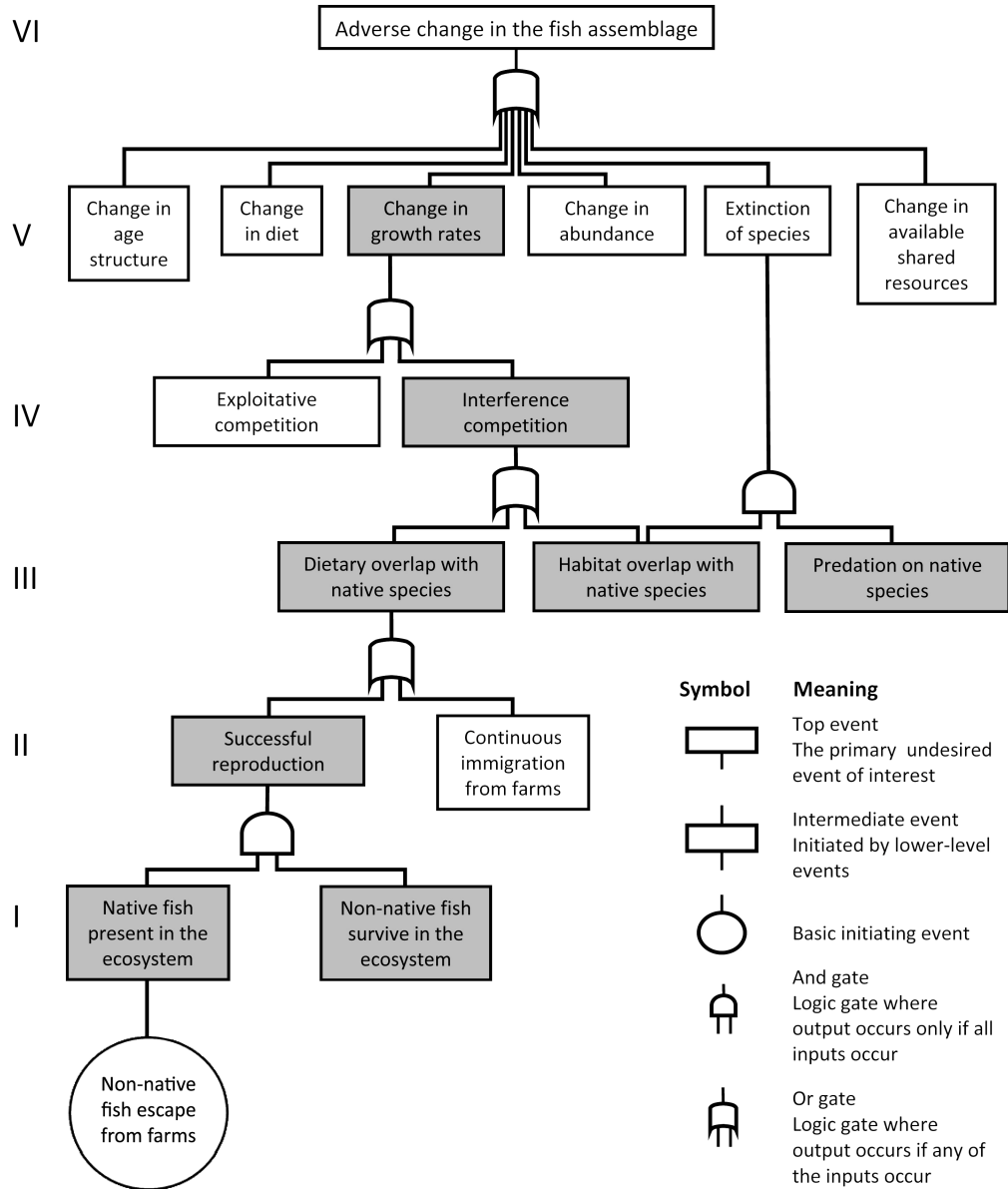
I wrote each chapter of this dissertation in the form of a manuscript to be submitted to a peer-reviewed journal for publication. Anne Kapuscinski and David Huff will be co-authors on the manuscript to be submitted for publication from Chapter 1, and Drs. Anne Kapuscinski and Wansuk Senanan will be co-authors on the manuscript to be submitted for publication from Chapter 2. Therefore, I use the plural voice in the chapters rather than the singular voice.

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**Figure 1.** A fault tree of events depicting exposure assessment endpoints (boxes at levels I – III) and effects assessment end points (boxes at levels IV – VI) in the ecological risk assessment (ERA) of a non-native fish escaping from aquaculture or intentionally released into an aquatic ecosystem containing native fishes. The fault tree should be read from the bottom to the top, starting with non-native fish escapees as the ecological stressor (circle) and ending with adverse change in the fish assemblage as the ecological response (event box at level VI). Levels V and VI consist of events affecting the native fish community. Assessment end points become more difficult to assess moving from the bottom to the top of the fault tree. Gray-filled boxes represent assessment endpoints for which this study obtained site-specific empirical data.



## **Chapter 1**

**Site, habitat and dietary overlap in small streams in the Eastern River system of  
Thailand: informing the ecological risk assessment of non-native  
*Oreochromis niloticus***

## Introduction

Diversity of freshwater fish is in crisis globally. Conservation efforts are hamstrung by a lack of basic knowledge of the biology and ecology of many fish species. What is known about the ecology infrequently influences the decisions made by resource users and policy makers. Freshwater ecosystems are vulnerable to cumulative impacts of multiple stressors, making the conservation of aquatic biodiversity difficult. Watersheds cross political and land use boundaries making it particularly hard to negotiate and implement conservation policies. Freshwater habitats are degraded by activities in the terrestrial realm. Habitat loss and non-native species are cited as the two greatest threats to freshwater fish biodiversity (Sala et al. 2000). In the temperate zone, extinction rates for freshwater vertebrates are five times higher than those for terrestrial vertebrates (Ricciardi and Rasmussen 1999). In the tropics, where diversity is greater, rates of loss are even higher and less is known about these ecosystems (Groombridge and Jenkins 2000; Loh 2000).

Southeast Asia, a hotspot of freshwater fish biodiversity, exemplifies these problems (*as reviewed in* Dudgeon 2003). The region has witnessed massive industrialization and deforestation. The human population is dense, largely rural, and dependent on freshwater fisheries for food. Many non-native fish species have been introduced. Understanding the effects of these factors on aquatic ecosystem function is difficult because few pristine systems remain to serve as a baseline and funding and facilities for research and monitoring are quite limited.

Freshwater species are a key source of animal protein for people in the developing world where the poorest peoples rely on wild-caught fish for food. In Lao People's Democratic Republic (PDR) and Cambodia, most animal protein comes from capture fisheries in the Mekong and Tonlé Sap rivers (Coates 2002). Thailand, a global hotspot for freshwater fish biodiversity, derives significant cultural, religious, economic, and dietary value from aquatic species. After World War II, *Oreochromis niloticus* aquaculture was widely introduced to Thailand and throughout the region to increase food security (Lever 1996; Welcomme 1988; Lowe-McConnell 2000). *O. niloticus* were also stocked in reservoirs and lakes to create wild fisheries. They are now farmed extensively in ponds and rivers for food, and Thailand is one of the top five producing countries (FAO 2007).

Potential environmental risks come with the societal benefits of freshwater aquaculture. For instance, Naylor et al. (2001) cited aquaculture as the largest pathway for the introduction of non-native species into aquatic ecosystems, and *O. niloticus* is a quintessential example of this. It has an extensive naturalized range covering parts of the Americas, Asia, Africa, Australia, and Europe, and feral populations are abundant relative to native species in several locations (Philippart and Ruwet 1982). Despite their widespread culture and establishment, only sporadic quantitative studies are available regarding the environments *O. niloticus* invade or their potential to affect native fishes. Their presumed effects, however, are mentioned throughout the literature (e.g. De Silva 2004).

Ecological risk assessment (ERA) provides a framework for asking key questions, collecting relevant data and communicating those data effectively to policy makers, so that society can make informed decisions regarding the conservation of biodiversity (Burgman 2005). The importance of ERA as a decision-support tool for biodiversity conservation is reflected in its inclusion in a number of high profile international agreements, such as the Convention on Biological Diversity (CBD 2000). The ERA framework can be used to inform environmental biosafety policy and regulation for aquaculture, particularly in developing countries (FAO 1995). Furthermore, there have been recent advances in the development and integration of methodologies to provide socially responsible and scientifically sound assessments of risks posed by introductions of aquatic organisms (Kapuscinski et al. 2007a).

If non-native fish escape to an environment that is able to support their survival and there are native fish present in this ecosystem (level I) this forms the foundation for species to interact. If level I of the fault tree (Figure 1, Prologue) is satisfied and there is establishment of the non-native (level II), from reproduction of feral fish or continuous immigration from farms, and the species share resources such as food and habitat (level III), the opportunity exists for competition to occur (level IV). Non-native escapees must be able to survive in their naturalized environment for an effect event to be realized. A working hypothesis in Thailand is that native piscivores, *Channa* spp., would prey on juvenile *O. niloticus* and therefore limit their invasive potential. However, in the face of continuous immigration of *O. niloticus* from fish farms, limiting natural recruitment

through predation of juveniles by a native species such as a *Channa* spp. may not be sufficient to limit the ecological effects of *O. niloticus*. This hypothesis, however, is based only on aquaculture pond studies and not natural ecosystems (Balasuriya 1988; Yi et al. 2002). There is a need to examine how environmental differences between aquaculture ponds and the wild; and the ability of these species to exhibit ontogenetic changes in diet might alter the interactions found in these studies. This requires spatially relevant dietary studies.

The aim of this study was to determine whether the ecology of *O. niloticus* in small streams in Thailand exposes native species to potential adverse effects. In the parlance of an ERA fault tree, this study focused on exposure events (levels II – III). Elsewhere, we report empirical research results focused on effects events (Chapter 2; levels IV – VI). Empirical results such as we report below are necessary to conduct a full ERA of a non-native fish species. In Thailand it was suggested that a native cyprinid, *Osteochilus melanopleura*, had experienced local extinctions after *O. niloticus* invasion in north central area of the country (Vidthayon, World Wildlife Fund Thailand, unpublished data). In addition, a fisheries station in southeastern Thailand has initiated a culture and stocking program for a native gourami, *Trichogaster trichopterus*, an important food and ornamental species, whose populations are thought to be in decline due to competition with feral *O. niloticus* (Bang Phra Fishery Manager, Thai Department of Fisheries, personal communication). Our objectives were to characterize the habitats and diets of feral *O. niloticus*, and to determine the extent of site, habitat, and dietary

overlap between feral *O. niloticus* and native fish species. Our study area consisted of small streams within the floodplain of *O. niloticus* farms and where *O. niloticus* stocking was not currently occurring. We measured 42 environmental parameters and characterized fish assemblages at 10 sites in the Eastern River system in southeastern Thailand. We analyzed ordinations to characterize the relationships among fish species, particularly species associations with *O. niloticus*. We also analyzed fish diets at *O. niloticus* sites to examine the extent of diet overlap in syntopic populations of *C. striata* and juvenile *O. niloticus*, and to document resource overlap with *T. trichopterus*.

## **Materials and Methods**

### Study area

Fieldwork was conducted during the dry season, from 20 - 23 December 2005, in small streams (1<sup>st</sup> - 4<sup>th</sup> order) at ten sites in the Eastern River system in southeastern Thailand (Figure 1). The study area is located in the lowland moist deciduous forest ecoregion within the tropical and sub-tropical moist broadleaf forest realm (Wikramanayake et al. 2002). The landscape is dominated by human activities, including terrestrial agriculture (palm oil, paddy rice, and sugar cane), freshwater aquaculture, and industrial operations. Streams are often channelized and serve as the main conduit for municipal and industrial effluent. We selected sites characterized by gentle gradients and a succession of shallow glides, pools, and riffles, from a pool of available and accessible sites within the floodplain of *O. niloticus* farms. All sites had water depth, clarity, and conductivity conducive to backpack electrofishing.

## Sampling

We assessed habitat characteristics and surrounding land use both quantitatively and qualitatively (Appendix 1). A site consisted of a length of stream of similar habitat. Each site was sampled once. The stream section length was recorded as the distance (m) between the upstream and downstream block nets. Width ( $\pm 0.05$  m) and depth ( $\pm 1$  cm) were each the mean of three measurements made at approximately equal intervals across the site. Discharge ( $\text{l s}^{-1}$ ) was calculated using the width, depth, and velocity ( $\pm 1$  cm) values. Velocity, measured at the surface, was adjusted to represent the mean flow rate (Gillner and Malmqvist 1998). The presence of pools, riffles, woody debris, and channelization were qualitatively assessed. Percent surface macrophyte cover and riparian cover were quantified visually. Elevation and site location were measured by GPS. Land-use activities (urban, industrial, terrestrial agriculture, animal agriculture, and natural lands) immediately surrounding each site were recorded as present or absent. Water chemistry parameters were measured with electronic meters. These included: pH ( $\pm 0.1$  unit), conductivity ( $\mu\text{S cm}^{-1}$ ), turbidity (NTU), dissolved oxygen ( $\pm 0.1$  mg  $\text{l}^{-1}$ ), and water temperature ( $^{\circ}\text{C}$ ). We used the salicylate method to measure ammonia (mg  $\text{NH}_3\text{N l}^{-1}$ ), FerroVer for total iron (mg  $\text{Fe l}^{-1}$ ), sulfuric acid titration for alkalinity (CaCO<sub>3</sub> mg  $\text{l}^{-1}$ , pH 4.5), cadmium reduction for nitrate (mg  $\text{NO}_3\text{N l}^{-1}$ ), and the heteroploy method for silica (mg  $\text{SiO}_2 \text{l}^{-1}$ ).

Substrate was evaluated visually. The diameter (mm) of surface particle size was assessed according to size categories adopted from the Wentworth scale (Gillner and

Malqvist 1998), >150 mm (boulder to large cobble), 150 - 60.1 mm (large cobble to large pebble), 60 - 5.1 mm (large pebble to coarse gravel), 5 - 3.1 mm (medium to fine gravel), 3 - 0.51 mm (fine gravel to coarse sand), and <0.5 mm (medium sand to silt). We visually estimated the percentage of primary and secondary particle sizes within sample quadrants (1 m<sup>2</sup>). Particle size categories were coded and the percentage of each, primary and secondary, was recorded.

Sites were electrofished by walking from the downstream block net to the upstream block net. Two to five passes were made per site. Seine nets (3 mm mesh size) with weighted groundlines were installed before sampling began, at the upper and lower limit of the sample site to reduce fish escapement from the sampled area. Conductivity was measured and used to set the voltage and wave configuration to minimize fish harm and maximize capture efficiency (Reynolds 1983). The length of stream sampled was based in part on the continuity of habitat and the physical constraints imposed by the environment (e.g. water velocity and discharge rate). After each pass, we identified each fish to species, measured its total length ( $L_T$ ; cm), and released it downstream of the block nets following the protocol of Beamish et al. (2006). We retained and euthanized a subset of captured individuals for gut content analysis. Due to collection permit limitations, we only collected: (1)  $L_T$  data from fish at sites containing *O. niloticus*; and (2) gut content data from species previously shown to have diet overlap with *O. niloticus* (Senanan unpublished data) at syntopic sites, namely *T. trichopterus*, *C. striata*, and *R. paviei*.



### *Fish assemblage and environmental data analysis*

We used a multipass removal methodology to obtain a maximum likelihood estimator (MLE) of species abundance ( $\hat{n}$ ) at each site (Otis et al. 1978; Rexstad and Burnham 1991; Zippin 1956). These estimates, and the area of stream sampled, were used to estimate species densities. Many species had few or zero individuals captured at each site, for these cases, we used  $n$  (number of individuals captured) in place of  $\hat{n}$ .

We used non-metric multidimensional scaling (NMS; PC-ORD, McCune and Mefford 1999) to examine association patterns of fish assemblage, species abundance, and environmental conditions among sampled sites. We ordinated fish assemblages in species space and correlated species abundance and environmental variables with the axes in an NMS ordination. The data were not normally distributed and contained many zero values; NMS is able to deal with such data in a robust and effective manner (Clarke 1993, McCune and Grace 2002). The ordination produces an increasing monotonic relationship between the intersample dissimilarity and ordination distance so that sites with more similar fish assemblages appear closer together in ordination space. This is also true for the species scores that are produced by abundance-weighted average site scores for each species. The optimal ordination solution is determined iteratively with departure from a perfect solution indicated by increasing stress (Kruskal 1964a). Low stress ordination solutions are defined by values  $< 2.5$  as excellent and  $< 5$  as good (Kruskal 1964b).

We performed two ordinations to examine differences in the associations of fish assemblages between groups of sites with and without non-native species. We coded sites as non-natives present or absent (hereafter, with/without non-natives). The first ordination included all species (natives and non-natives). The second ordination did not include non-native species (see Table 1 for the names of non-native species). We excluded rare species which occurred at only one site (Site 2342;  $n = 12$ ) from both ordinations.

In both ordinations, we used the Euclidean distance measure and a random starting configuration with 250 runs of real data (Sneath and Sokal 1973). We applied a Bray-Curtis correction (i.e. natural log  $(1 + x)$  transformation), due to the large number of low frequency species, to the raw data matrices comprising the numerical abundance of 21 species in the first ordination (19 species in the second ordination) from 10 sample sites. This correction also shifted the emphasis of the analysis to proportions of species per site rather than absolute abundance. We applied this transformation to all variables in the environmental matrix except pH. Dimensionality was assessed using a 0.000000 stability criterion (i.e. standard deviations in stress over the previous ten iterations). Monte Carlo simulations, with 250 randomized iterations, were run to select the best dimensionality ( $p \leq 0.05$ ) in each ordination.

We used several methodologies to visualize and test for differences in the fish assemblage and species data based on the categorical grouping variable: with/without

non-natives. We examined the indicator value of individual species for separating the groups with Dufrêne and Legendre's (1997) indicator species analysis. Then, we used the Multiple Response Permutation Procedure (MRPP) to compare the fish assemblage at sites in each group (Mielke 1984; Mielke and Berry 2001). This is a nonparametric procedure for testing our hypothesis that there was no difference between sites with and without non-natives. In addition, we calculated standardized chi-squared distances for species associations, excluding associations with expected values  $< 5$  to examine the level of association between these species. We compared the positions of the ordinated species values (i.e. weighted averages) on the ordination axes with the environmental variables using the squared correlation coefficients ( $R^2$  and tau). The squared values of the correlation coefficients express the proportion of variation in position on an ordination axis that is explained by the environmental variable in question. We used the environment matrix with the assemblage and species data from the first ordination (i.e. non-native abundances included), both correlation measures, the total absolute tau and the  $R^2$  values, because the distribution of some binary (i.e. present/absent) variables among sites was not normal (e.g. urban land use, Shapiro-Wilkes test  $p = 0.208$ ). Lastly, we used joint plots to display the relationships between environmental variables, species values, and the ordination axes.

#### Fish diet analysis

Fish ( $n = 62$ ) were taken on ice from the field to the laboratory where they were measured ( $L_T$ , cm and weight, g), and their guts were removed. The anterior third of the

intestine of cyprinids was dissected because they lack a discrete stomach. Gut contents were preserved in 95% ethanol. Specimens lacking food in the stomach (anterior third in cyprinids) were categorized as empty, and those containing food were classified as to their degree of fullness. Gut contents were categorized into ten diet categories based on the groupings proposed by Njiru et al. (2004) and Nithirojapakdee et al. (2007). Diet categories were: fish remains, crustaceans, mollusks, aquatic insect larvae, adult terrestrial insects, benthic oligochaetes, digested plant material, freshwater sponges (hydra and bryozoa), zooplankton, and phytoplankton.

#### *Data analysis*

We calculated two measures of diet: proportion (P) and frequency (F). Hynes' (1950) point method was adapted to calculate P as each diet category's proportion of the total volume of diet categories within an individual's stomach. We used a stereo microscope to make a rough estimate of the P of each diet category, especially for large prey items. Then we used a compound microscope to fine tune the former estimation and to examine debris unidentified by the stereo microscope at x40, x100, and x400 magnification. We estimated P based on the approximate size of live organisms rather than on the size of the remains in the gut. This accounted for diet types that were more easily digested, e.g., oligochaete setae represented an entire individual and a whole oligochaete accounted for a larger proportion of the stomach compared to phytoplankton or zooplankton. We calculated mean P ( $\bar{P}$ ) of each diet category for each species captured at a site. The frequency (F) of stomachs containing a diet category, or

occurrence of diet category, for each species at a site (Hynes 1950; Williams 1981) was calculated as,

$$F_i = (F_i \times 100) * S^{-1},$$

where  $F_i$  is the number of guts, by species, containing diet type  $i$ , and  $S$  is the total number examined.

We used two indices to examine dietary overlap and to identify and compare the relative importance of diet categories between species captured at the same site (sites 2012 and 2341) and on a spatial scale among sites for individual species. Dietary overlap ( $O_D$ ) between different species or sites was compared using the index proposed by Schoener (1970),

$$O_D = 1 - (0.5 \sum |P_a - P_b|) 100^{-1},$$

where  $P_a$  is the percentage of a food item in species  $a$ , and  $P_b$  is the percentage of a food item in species  $b$ . This index produces values between 0 (no overlap) and 1 (complete overlap). The relative importance of food items was evaluated using the index of food importance ( $I$ ) (Herder and Freyhof 2006),

$$I = 100 FP (\sum FP)^{-1},$$

where  $F$  = % occurrence and  $P$  = % volume (Appendix 2).

## Results

*O. niloticus* were present and abundant in the Eastern River system and they were likely reproducing there as well. Most other species ( $n = 31$ ) were rare and present at very low relative abundance, and few species were highly abundant (Table 1). *O. niloticus* were captured at three of ten sample sites and were the most abundant species (sites 2012 and 2341) or highly abundant (Site 2011), comprising, 81.19, 58.10 and 16.92% of the fish captured at these sites. Across all sites, *O. niloticus* accounted for 23.45% of all sampled individuals, more than any other species (Appendix 3). Sizes of *O. niloticus* ranged from 1 to 10 cm, with an average size of  $2.97 \text{ cm} \pm 0.06 \text{ SE}$  (Table 2).

### Native fish assemblage

We found that the fish assemblage at sites where non-natives occurred was different from sites that consisted only of native species. Other non-natives ( $n = 2$ ) were present only at *O. niloticus* sites and at very low abundances (Table 1). The first ordination solution, where non-native fish abundances were included, was 3-dimensional with low stress, 1.07458, after 60 iterations. The proportion of the variance represented by the axes, based on the correlation between the distance in ordination space and the distance in original space, was 95.1%. In this ordination, fish assemblages at sites with non-natives clustered together, further along each axis, than those at sites without non-natives (Figure 2a). Cluster analysis supported this grouping with 70% chaining (Figure 2b), and the MRPP found that the fish assemblage at sites with non-natives was significantly different from the assemblages at sites without non-natives ( $p = 0.0425$ ). In

addition, *O. niloticus* was the only significant indicator species (Monte Carlo test  $p = 0.0094$ ) of the fish assemblage at non-native sites.

The distribution of sites within the ordination changed in the second ordination when non-native abundance values were removed from the matrix (second ordination solution). This ordination solution was two dimensional with low stress, 5.16872, after 61 iterations. The proportion of the variance represented by the axes was 94.9%. Sites with non-natives no longer clustered apart from sites without non-natives (Figure 2c), and no significant difference in the native fish assemblage was found between sites with and without non-natives (MRPP  $p = 0.7900$ ).

### Environment

The distribution of species with respect to highly significant habitat variables showed that all non-native species clustered near each other and apart from native species, at the far reaches of the habitat gradients (Figure 2d). Overall, species gradients were correlated with a wide range of environmental variables. Most environmental variables had high total  $R^2$  ( $\geq 0.2$ ) and tau values across all axes (Appendix 1). Stream channelization and urban and agricultural land use followed a strong non-native species gradient. The presence of woody debris and cover (riparian and emergent macrophyte) followed a strong native species gradient (Figure 2d), from which *O. niloticus* and other non-natives grouped apart.

### Resource use overlap

We found that juvenile *O. niloticus*, *C. striata*, and *T. trichopterus* are syntopic at our study sites, are highly associated with one another, and rely on shared resources. *C. striata* was not a relevant predictor of fish assemblage as they occurred throughout the study area (9 out of 10 sites) and at all *O. niloticus* sites ( $n = 3$ ) (Table 1). The ordination showed that *C. striata* was not an important indicator of the presence or absence of *O. niloticus*, and standardized chi-squared distances showed that *O. niloticus* and *C. striata* are positively associated with one another (plexus = 0.2182). Plexus values range from -1 to 1, with +1 indicating perfect positive association and -1 a perfect negative association (McCune and Mefford 1999). *O. niloticus* and *T. trichopterus* also co-occurred and were positively associated with one another (plexus = 0.2182). *T. trichopterus* were present at all *O. niloticus* sites and the two species were often collected simultaneously from small pools.

We found that diet of these species was consistent among sites (Appendix 2). We did not find evidence that *C. striata* prey on juvenile *O. niloticus*; rather, the two species rely on shared food resources (Table 3; Figures 3a, 3b and 3c). We found little variation in the importance of diet categories for *C. striata* on a spatial scale, and dietary overlap was high among sites (Figure 3c). Adult insects and oligochaetes were the most important diet categories for *C. striata* and *O. niloticus*, and fish was relatively unimportant for either species' diet. High Schoener Index values (Site 2012  $O_D = 0.6136$  and Site 2341  $O_D = 0.7950$ ) for *O. niloticus* and *C. striata* indicate that both species rely



on many of the same food resources (Table 3). Gut content analyses of *T. trichopterus* and *O. niloticus* from the same site revealed a high degree of dietary overlap (Site 2012  $O_D = 0.8206$  and Site 2341  $O_D = 0.7725$ ), with adult insects and oligochaetes as the most important diet categories for both species (Table 3; Figures 3a, 3b, 3d and 3e) among sites. We also found little variation in *O. niloticus* diet and high overlap across sites (Table 3; Figure 3e).

## Discussion

We organized our research around an ecological risk model, consisting of a fault tree of events necessary for a non-native fish species (the ecological stressor) to cause an adverse change in native fish assemblages (the ecological response). Field research is typically needed to obtain data for use in exposure assessment (events at levels I-III) and this study is an illustrative example. Manipulative experiments are typically needed to determine causation—whether and how the stressor causes an adverse effect and to obtain data for effects assessment (levels IV-VI); and we report elsewhere such work for our study system (Chapter 2). Our results show that Thai native fish assemblages are exposed to the ecological stressor because *O. niloticus* individuals are highly abundant relative to native species, are likely reproducing in the wild, and have high habitat and dietary overlap with selected heterospecifics. Furthermore, the presence of piscivorous *Channa* spp. does not occlude the presence of juvenile *O. niloticus*. In ERA parlance, *Channa* spp. do not reduce exposure to the stressor. Rather, these species are associated with one another and rely on shared resources. Our results illustrate the

types of data and analyses that risk assessors will need in order to quantitatively estimate the probability and magnitude of establishment and resource overlap in a site-specific ERA of any introduced fish.

Before our study, quantitative ecological data on *O. niloticus* in Thailand were severely limited to a few data sets on their abundance in Thai reservoirs and major rivers in Northeast Thailand (De Silva 2004; Dumrongtripob, Thai Department of Fisheries, unpublished data). Such data can inform estimation of establishment (level II) but cannot inform estimation of resource overlap (level III) and ecological effects (level V; Moyle and Light 1996). We found high levels of habitat and dietary overlap between *T. trichopterus* and *O. niloticus*, which could lead to competition between these species and potential ecological effects (levels IV, V, and VI). We also found that *O. niloticus* do not prey on native fishes (level IV and Figure 3e). Our research therefore starts to fill a critical gap in the ecological information needed for a quantitative exposure assessment.

Our results suggest that *O. niloticus* readily become feral and are likely reproducing in the wild in Thailand. Although our sampled sites were within the floodplain of *O. niloticus* farms, we did not capture adult escapees. Instead, the size range of captured individuals ( $L_T \leq 10$  and  $L_T \bar{2}.97$ ) corresponds to the juvenile life stage and indicates that these fish were likely hatched in the wild from feral parents. The individuals we captured probably descended from fish farm escapees although we

cannot rule out the possibility they descended from intentional releases by the Thai Department of Fisheries conducted to support subsistence fisheries. The high abundance of juvenile *O. niloticus* (23.45% of all fish sampled across all sites) suggests successful establishment of the species at our study sites. Because an adverse change in the fish assemblage is conditional on the probability of lower level events in a fault tree, this high abundance could contribute to a high overall risk of adverse change in the fish assemblage (Lockwood et al. 2005; Marchetti et al. 2004). Ruling out this risk would require a counteracting mechanism, such as high predation on *O. niloticus* juveniles, to preclude or greatly reduce the probability of resource overlap or competition (levels III and IV, respectively). Risk assessors need to expand data collection beyond our study, in order to quantify establishment of *O. niloticus* over larger areas of relevant water bodies. Alternatively, to better use their limited resources, risk assessors could assume a 100% probability of *O. niloticus* establishing in suitable habitats and focus on data collection needed to quantify the probability and magnitude of subsequent events in the fault tree.

Our results do not support the hypothesis that predation by *Channa* species in Thai waters will prevent exposure of native fish assemblages to possible adverse changes from *O. niloticus*. Before *O. niloticus* were introduced to Thailand, fisheries managers assumed that piscivory by *C. striata* would significantly limit the recruitment of feral *O. niloticus*. A study in natural systems in western Thailand shows that *C. limbata* are highly piscivorous (Ward-Campbell and Beamish 2005). Experiments in

aquaculture ponds showed that *Channa spp.* are piscivorous and completely control *O. niloticus* recruitment in these artificial systems (Balasuriya 1988; Yi et al. 2002). We sampled two *Channa* species, *C. striata* and *C. limbata*, and analyzed diets of the former, which was syntopic with *O. niloticus*. Although the *C. striata* captured in our study were much larger and thus more likely to prey on fish (Table 2) than the *C. limbata* sampled by Ward-Campbell and Beamish (2005), fish had a low relative importance in their diet (Appendix 3, Figures 3a, 3b, and 3c). Dietary overlap ( $O_D$ ) between *O. niloticus* and *C. striata* was high for individuals sampled at the same site (Figure 3a and b) and among sites for both species (Figure 3c). In addition, these species were positively associated with one another. Therefore, the dietary preferences of *C. striata* did not limit the distribution of juvenile *O. niloticus*, as previously presumed.

The conduct of any ERA for *O. niloticus* should require site-specific evidence and quantification of piscivory on feral individuals and not assume that predation will reduce the probability or magnitude of adverse ecological effects. It is equally important to research whether and how *O. niloticus* might alter native fish assemblages through mechanisms of interference or exploitative competition (level IV). Published reports on *O. niloticus* introductions and the decline of native fishes largely assume that *O. niloticus* outcompete native species (Ogutu-Ohwayo 1990). If future empirical studies show that feral *O. niloticus* can adversely alter fish assemblages in Thailand, the conduct of any full ERA should quantify this risk and aquaculture regulators should consider risk management measures to limit further release of fish from existing farms.

The native fish assemblages at sites where *O. niloticus* have successfully invaded were not significantly different from those at un-invaded sites. The apparent difference between sites with and without non-native species may be attributable to the presence or absence of non-natives and not to differences in the native fish assemblage *per se*. Our results show that, when non-native abundance values were removed from the ordination, there were no significant differences among the fish assemblages at sites with/without natives. Indicator species analysis supports this as well—no taxon acted as a strong indicator of sites with/without non-native species besides the non-native species themselves. Perhaps other anthropogenic stressors had degraded the native fish assemblages, with extirpation of sensitive species prior to the arrival of *O. niloticus*; and after arrival, *O. niloticus* did not further alter the degraded fish assemblage significantly. Comparisons of the species ordination values with the environmental correlation coefficients revealed that non-native species exist at the far end of strong habitat gradients representing degraded conditions. Most of our study sites represented highly disturbed streams used as the main conduit for municipal and industrial waste. The broad consequences of large nutrient and contaminant loadings on habitat quality alone may have degraded the fish community at all sites. The one sampled site (2342) that was surrounded by natural lands had higher fish diversity, with many species only found at that site, and absence of non-native species. This site could lack *O. niloticus* because of invasion resistance by a more intact native fish community, its relative lack of disturbance, or because *O. niloticus* have simply not yet arrived (Moyle and Light 1996;

Tilman 1994). If *O. niloticus* were to invade this site, which has fewer anthropogenic stressors, it would be important to monitor whether adverse changes in the fish assemblage occur.

An approximate timeline and history of the *O. niloticus* invasion in the study area would aid interpretations of our results. Typical of many invasive species problems, we lack the most basic temporal information, such as when *O. niloticus* first became feral in the study area. Perhaps they have only had the opportunity to invade three sites thus far, or have invaded and gone extinct at some sites. Particularly sensitive species may have gone extinct following invasion but prior to our study. Alternatively, some sensitive species in the invaded sites may not have gone extinct yet, but may do so in the future. This is why numerous risk assessment researchers have called for targeted collection of baseline ecological data (Kapuscinski et al. 2007b; Lodge et al. 2006).

*T. trichopterus* is a valuable species in capture fisheries and aquaculture, both for human food and the ornamental aquarium trade. A previous study showed that *O. niloticus* and *T. trichopterus* have dietary overlap within and among large reservoir wetland systems in Thailand (Senanan, Burapha University Thailand, unpublished data). We found that, in addition to dietary overlap, these species share habitat with *O. niloticus* in small streams. We sampled both species simultaneously from the same small pools and their dietary overlap was high within and among sites (Figures 3a and 3b). We

also noted the potential for reduced abundance of *T. trichopterus* at *O. niloticus* sites (level V), which could result from competition.

Site-specific field research, as reported here, should provide the data for quantitative exposure assessment of fish introductions, an essential component of a full ERA. Methodologies for quantitative exposure and effects assessment of non-native species and introduced genotypes, such as selectively bred or transgenic lines, have been rarely used (Kapuscinski et al. 2007a), with major gaps in key ecological information being one of the impediments. Researchers can develop ERA conceptual models for their study systems, such as the fault tree used in this study, to help identify the most important ecological research to conduct and thus better target limited resources to more quickly fill policy-relevant information gaps.

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**Table 1.** Spatial survey of relative fish abundance (%) at sites (n = 10) within the Eastern River system in December 2005. Sites containing non-native species represented by filled circles and sites with only native species represented by open circles in all cases. Abbreviated names provided for each species, and asterisks denote non-native species. Total number (n) of fishes caught per site provided at bottom of each column.



Table 1

Genus and species	Species	Site											
		2011	2012	2013	2013	2123	2231	2232	2233	2341	2342	2343	
<i>Anabus maspembelus</i>	A.mas	●	○	○	○	○	○	○	○	○	○	○	○
<i>Anabus testudineus</i>	A. tes			16.7	13.3								2.0
<i>Hemibagrus nemurus</i>	H.nem			7.3	1.2			3.6					16.3
<i>Leiocassis siamensis</i>	L.sia												8.2
<i>Mystus mysticetus</i>	M.mys			0.4			13.0						0.0
<i>Tylorus acus melantus</i>	T.acu											2.0	
<i>Channa limbata</i>	C.lim							19.3				4.1	14.7
<i>Channa striata</i>	C.str	1.4	7.9	5.5	22.9	1.9	10.8	17.6	5.4				23.5
<i>Oreochromis niloticus*</i>	O.nil*	15.7	81.2						58.1				
<i>Clarias batrachus</i>	C.bat			0.4	1.2	0.6	2.4						8.8
<i>Chela laubuca</i>	C.lau												2.0
<i>Esomus metallicus</i>	E.met			31.3	7.2	32.3		70.6	20.0				
<i>Hypophthalmichthys molitrix*</i>	H.mol	1.4											
<i>Osteochilus hasselti</i>	O.has			0.4									4.1
<i>Labiobarbus leptocheilus</i>	L.lep			0.4		0.6							2.0
<i>Rasbora borapetensis</i>	R.bor												
<i>Rasbora paviei</i>	R.pav	52.9	0.0	12.7				36.1					8.2
<i>Lobocheilus sp.</i>	Lobo												14.3
<i>Systemus binotatus</i>	S.bin	1.4											8.2
<i>Systemus martitus</i>	S.mar												4.1
<i>Rhinogobius chiengmaiensis</i>	R.chi												4.1
<i>Dermegetys pusillus</i>	D.pus												
<i>Mastacembelus erythrotaenia</i>	M.ery						1.2						2.0

Table 1 continued

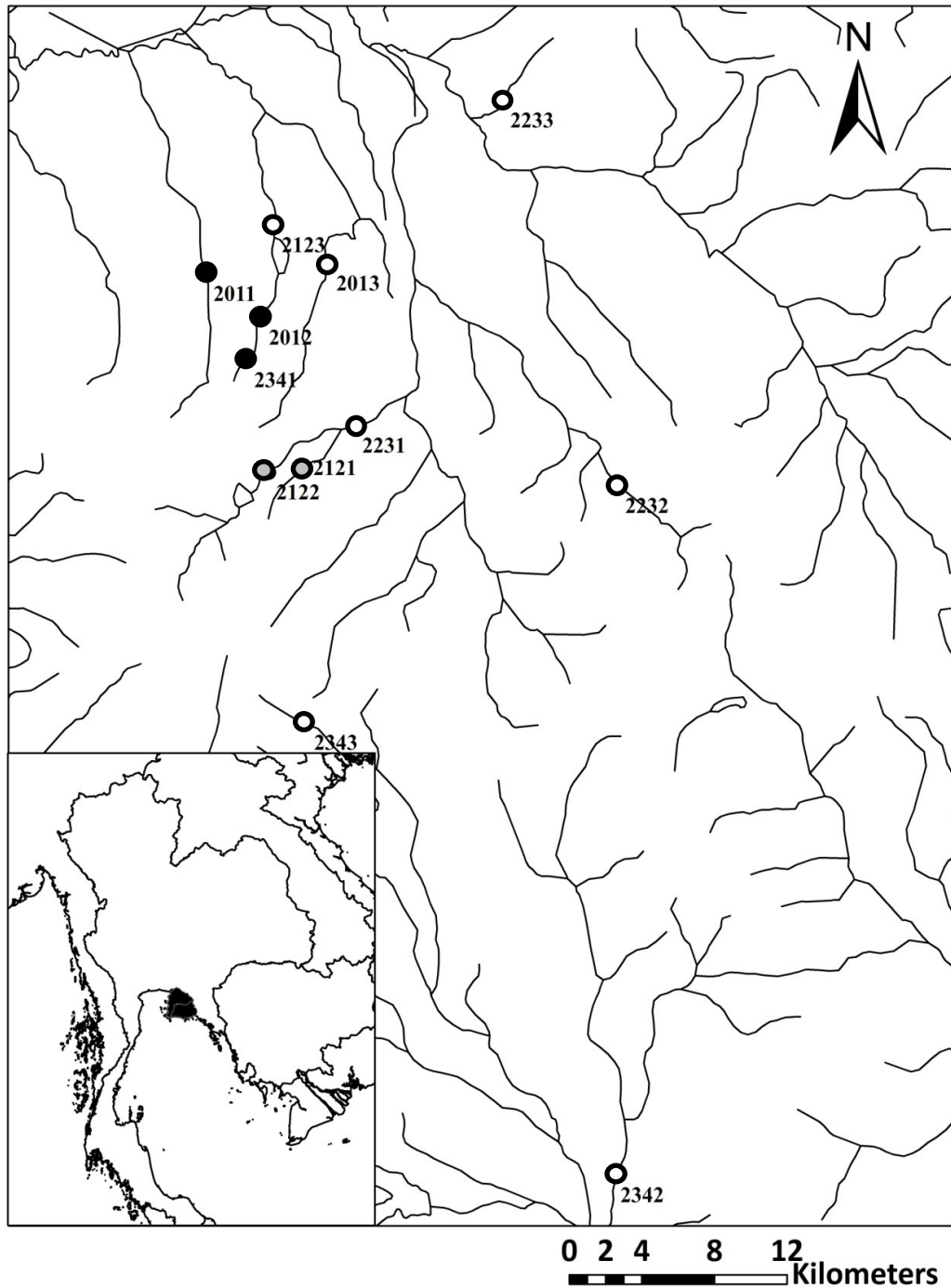
Genus and species	Species	Site											
		2011	2012	2013	2013	2123	2231	2232	2233	2341	2342	2343	
<i>Mastacembelus armatus</i>	M.arm												2.0
<i>Gnathonemus petersii</i>	G.pet												4.1
<i>Gambusia affinis holbrooki</i>	G.chu												4.1
<i>Trichogaster trichopterus</i>	T.tri	18.6	4.0	22.9	49.4	44.1	4.8		14.3	6.1			2.9
<i>Trichopterus vittatus</i>	T.vit	7.1	4.0	1.8	4.8	5.6			1.6				
<i>Poecilia reticulata</i> *	P.ret*		3.0										
<i>Ompok bimaculatus</i>	O.bim			0.4		1.2							
<i>Monopterus albus</i>	M.alb	1.4				0.6		11.8		2.0			29.4
Total n		70	101	275	83	161	83	34	315	49			34

**Table 2.** Total length ( $L_T$ , cm) of captured fishes at selected sites (denoted by an “x”). Columns report the number (n) of fishes measured, their mean  $L_T$ , one standard error of the mean (SE), and size range.

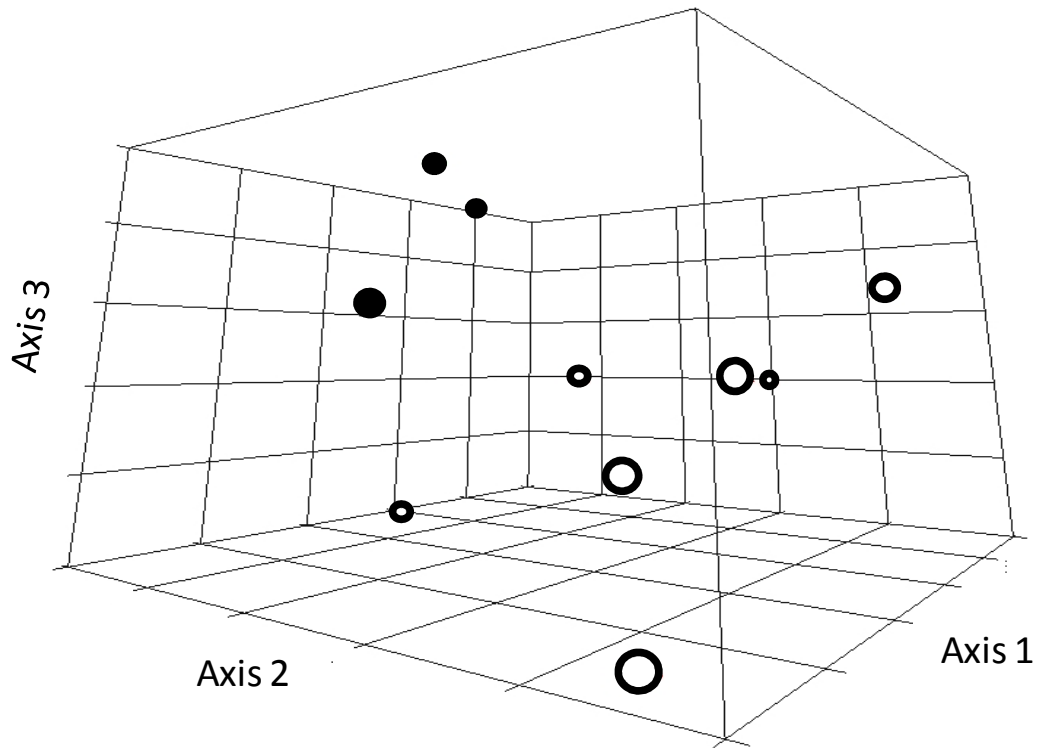
Species	n	Sites				Size Range			
		● 2011	● 2012	○ 2121	● 2341	Mean	SE	Minimum	Maximum
A. tes	1				x	19.50		19.5	19.5
C. str	31	x	x	x	x	27.86	4.08	9.8	106.1
C. bat	1				x	1.32		1.3	1.3
H. mol*	1	x				4.50		4.5	4.5
M. alb	1	x				27.00		27.0	27.0
O. nil*	279	x	x		x	2.97	0.06	1.0	10.0
P. Ret*	2		x			2.50	0.20	2.3	2.7
R. Pav	96	x			x	4.04	0.06	2.0	5.2
T. tri	61	x	x		x	6.04	0.18	2.5	9.0
T. vit	13	x	x		x	2.23	0.42	0.2	4.5

**Table 3.** Pair-wise comparisons of dietary overlap as represented by the Schoener-Index ( $O_D$ ), ranging from 0 = no overlap to 1 = total overlap (Schoener 1970), between species at site 2012 and site 2341 and between sites for species, *C.str*, *T.tri*, and *O.nil\**.

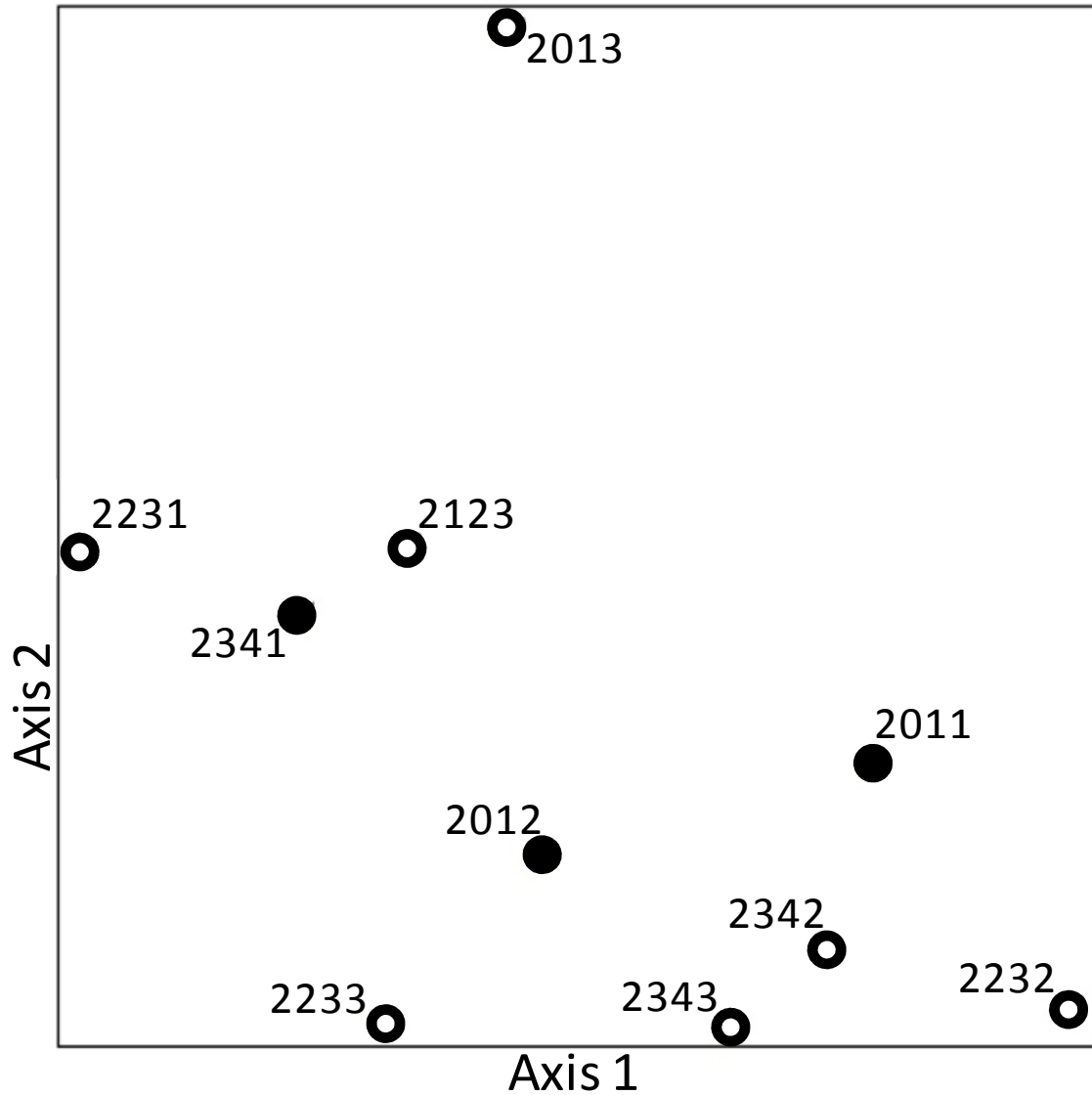
Species				Sites				
Site	$O_D$	T. tri	O. nil*		$O_D$	2012-2341	2011-2341	2011-2012
2012	C.str	0.7403	0.6136	Species	C.str	0.6335	0.7820	0.6138
	O.nil*	0.8206			T.tri	0.7557		
2341	C.str	0.8175	0.7950		O.nil*	0.8262		
	O.nil*	0.7725						



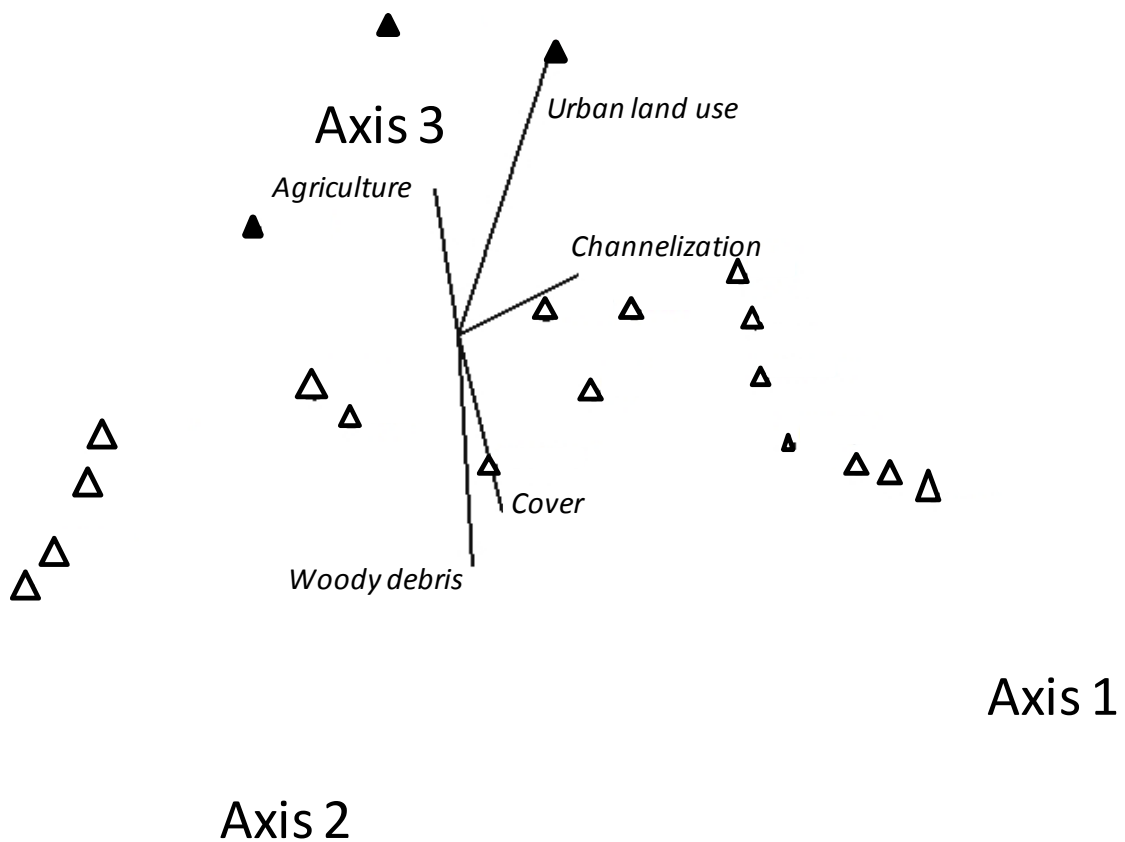
**Figure 1.** Map of the study area and sites (circles) in the Eastern River system (inset). Sites containing non-native species represented by black-filled circles, sites with only native species by open circles, and sites with no fish by gray-filled circles. The study area was bounded to the north and south at approximately  $101^{\circ}11'$  and  $101^{\circ}32'$  longitude and to the east and west at  $12^{\circ}90'$  and  $13^{\circ}43'$  latitude.



**Figure 2a.** Nonmetric multidimensional scaling (NMS) ordination of 10 sites in fish assemblage space. Filled circles represent sites with non-native species and open circles represent sites with only native species. Two ordinations were run to examine differences in the fish assemblage between groups of sites where non-natives were present and absent. In this ordination all species (natives and non-natives) were included, resulting in a three-dimensional solution with non-native sites (2341, 2012, and 2011) located at the furthest distance along the axes relative to the other sites.

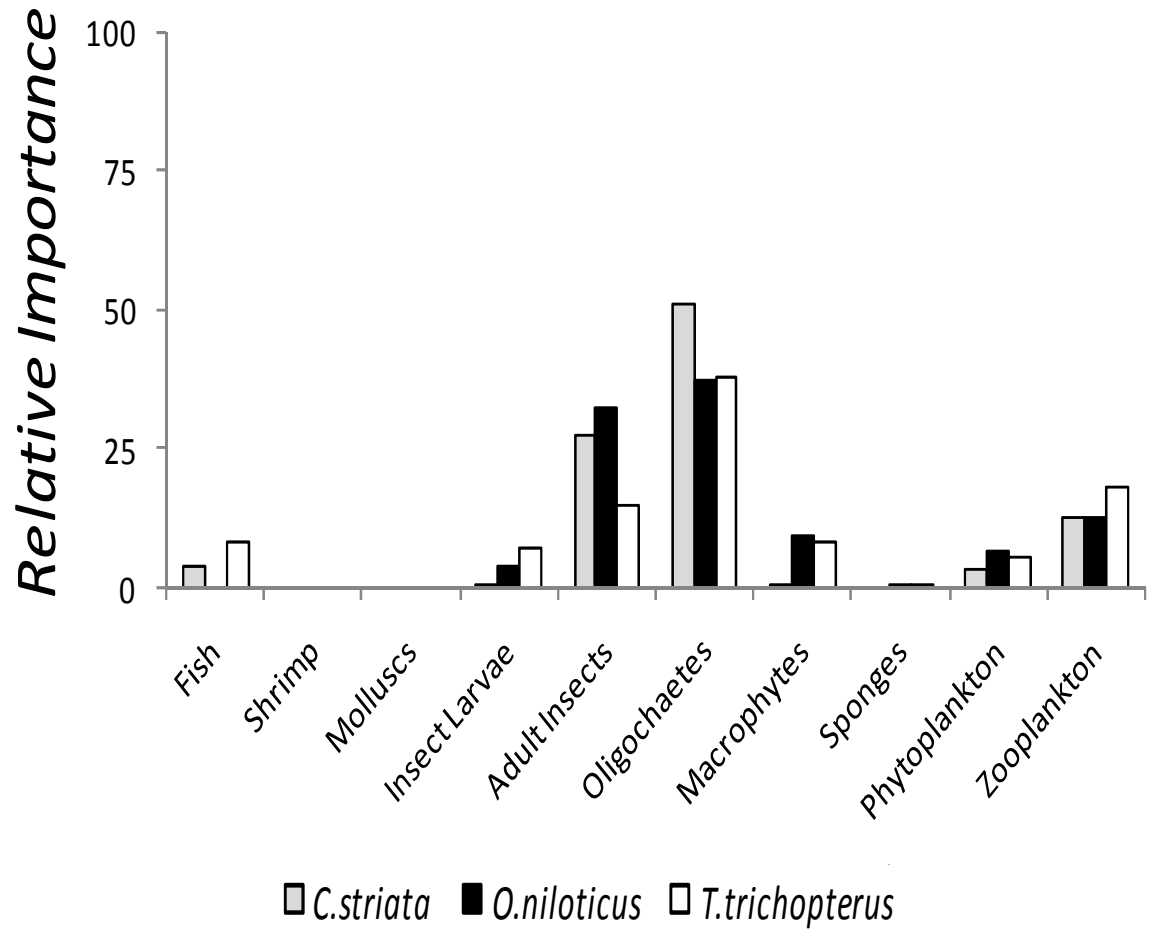


**Figure 2b.** Nonmetric multidimensional scaling (NMS) ordination of 10 sites in fish assemblage space. Filled circles represent sites with non-native species and open circles represent sites with only native species. Two ordinations were run to examine differences in the fish assemblage between groups of sites where non-natives were present and absent. When non-natives were excluded, this two-dimensional ordination solution resulted with no apparent clustering of sites.

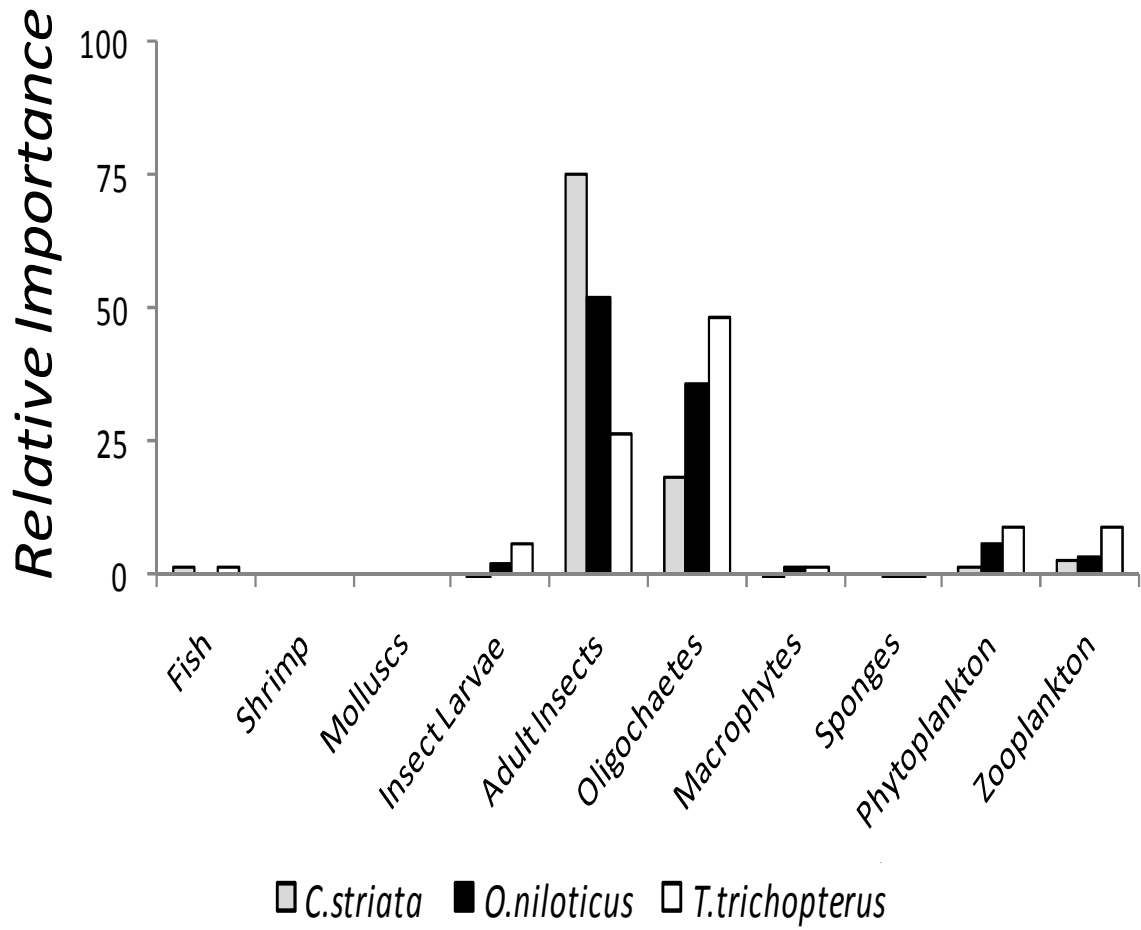


**Figure 2c.** Nonmetric multidimensional scaling (NMS) ordination of 10 sites in species space. Filled triangles denote non-native species and open triangles denote native species. All species (natives and non-natives) were included in this ordination, which resulted in a three-dimensional solution. The angles and lengths of the radiating lines indicate the direction and strength of relationships of the species and habitat variables. Highly correlated environmental variables, land use (agricultural and urban) and channelization followed a strong non-native species gradient, and the presence of woody debris and cover (riparian and emergent macrophyte) followed a strong native species gradient.

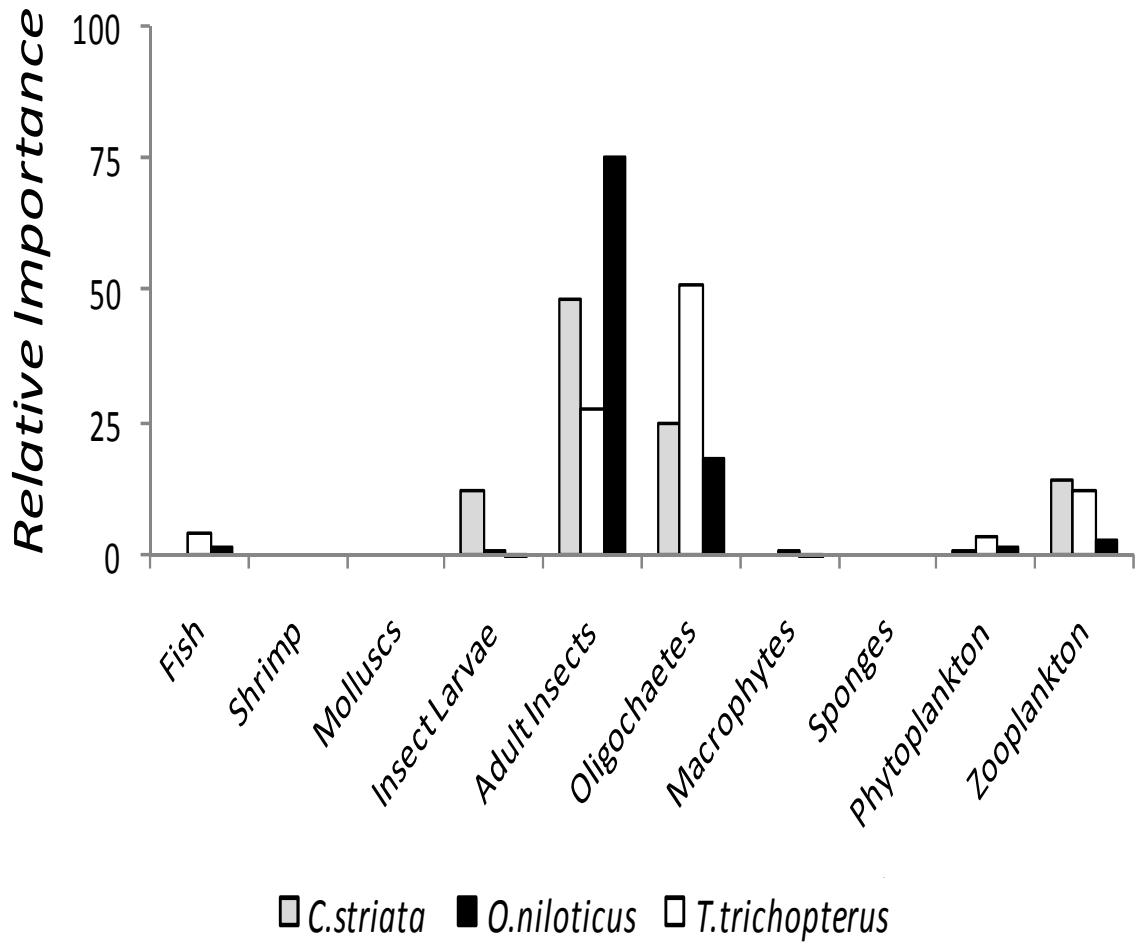




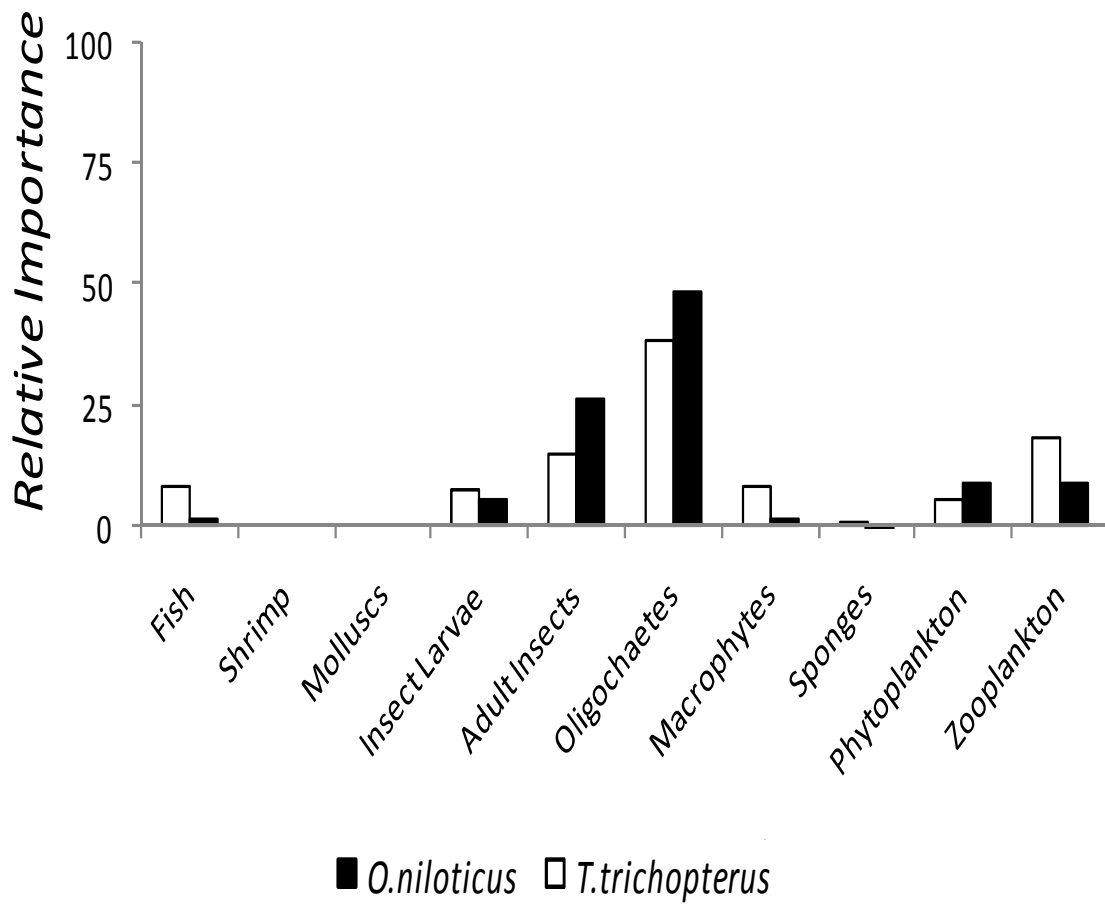
**Figure 3a.** Relative importance (*I*) of diet categories for species at site 2341.



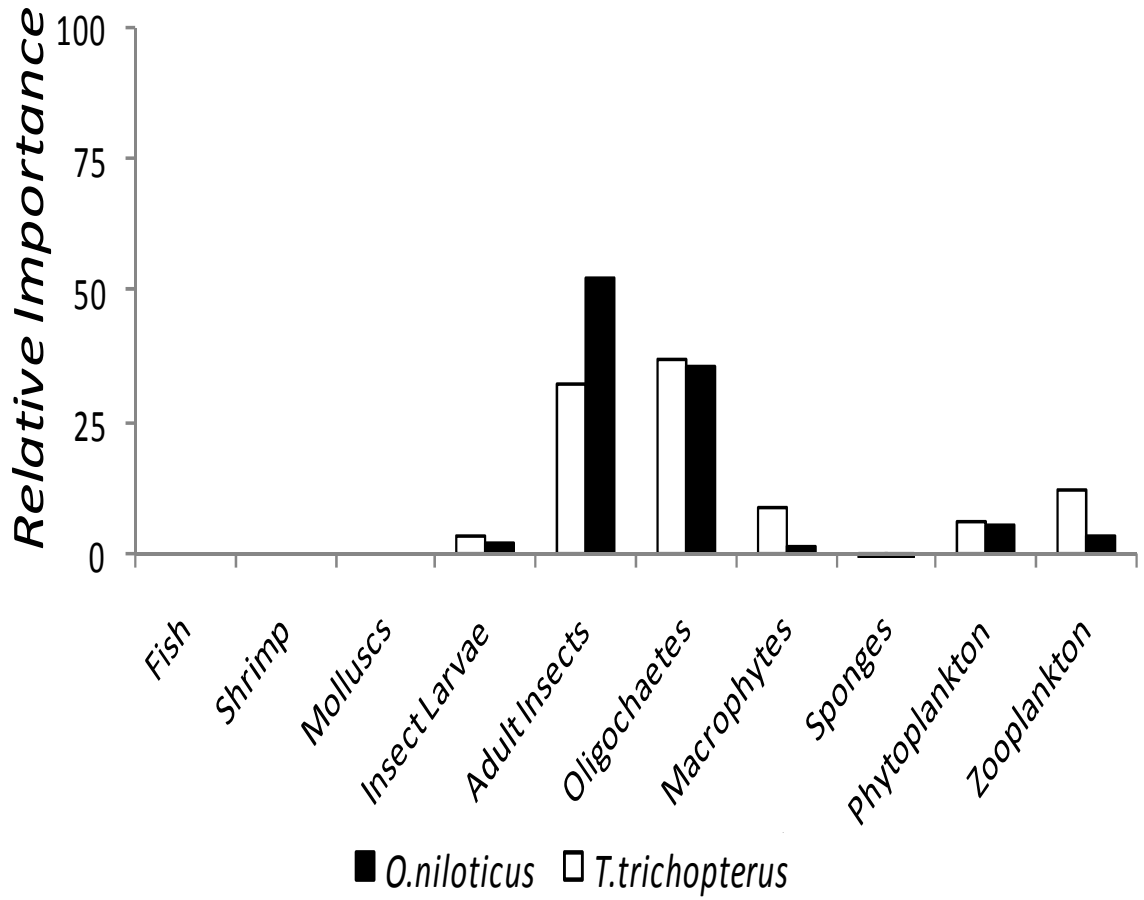
**Figure 3b.** Relative importance (*I*) of diet categories for species at site 2012.



**Figure 3c.** Spatial comparisons of the relative importance (*I*) of diet categories for *C.str* between sites.



**Figure 3d.** Spatial comparisons of the relative importance (*I*) of diet categories for *T.tri* between sites.



**Figure 3e.** Spatial comparisons of the relative importance (*I*) of diet categories for *O. nil\** between sites.

**Appendix 1.** Environmental and habitat variables for each site, and their Pearson and Kendal Correlations (total  $R^2$  and tau) for the 3-dimensional ordination. Some variables were recorded as present/absent (P/A) at each site.



Appendix 1 continued

Variables measured	Total R <sup>2</sup>	Total tau	Sites										
			2011	2012	2013	2013	2123	2231	2232	2233	2341	2342	2343
Volume sampled m <sup>3</sup>	0.192	0.299	24.1875	10.3075	28	10.89333	5.270833	8.791111	19.5	17.94	336	15.46667	
Stream order	0.189	0.479	3	2	2	3	1	1	2	1	4	2	
Substrate 3.1 to 5 mm	0.181	0.687	0	0	0.1	0	0	0	0	0	0.8	0	
Silica mg/L	0.166	0.495	1.81	1.4	1.81	1.24	1.13	1.34	1.78	1.86	1.58	1.37	
Nitrite mg/L	0.165	0.296	0.004	0.144	0.385	0.083	0.005	0.004	0.385	0.8	0.9	9.4	
Elevation m	0.159	0.315	50	44	35	16	31	31	15	14	7	113	
Flow cm/second	0.144	0.599	39.46	2.52	7.13	4.91	3.45	5.4	6.38	3.9	4.42	4.53	
Area sampled m <sup>2</sup>	0.139	0.333	58.05	99.75	87.5	86	68.75	61.33333	195	156	420	145	
Phosphate mg/L	0.115	0.378	2.8	2.6	1.1	2.2	0.46	0.43	3.4	0.29	1.18	0.02	
Total Iron mg/L	0.109	0.689	0.65	0.52	0.23	0.44	0.76	0.38	2.5	3.87	4.42	0.42	
Alkalinity pH 4.5 mg/L	0.108	0.270	220	180	250	118	74	170	238	174	180	30	
Alkalinity pH 4.8 mg/L	0.102	0.244	200	170	236	112	70	156	228	162	166	22	
Average Stream width m	0.100	0.111	2.15	2.216667	3.5	2	1.25	1.533333	3.25	2.6	6	2.9	
Ammonia mg/L	0.098	0.270	0.39	0.3	0.88	0.19	0.19	0.23	1.1	0.62	0.59	2.04	
Substrate 0.51 to 3 mm	0.090	0.516	0.2	0.1	0	0.9	0	0.8	0.8	0.9	0	0	
Turbidity NTU	0.077	0.289	15.2	28.2	47.6	31.7	19.1	17.9	24.6	10.7	15.6	73	
Substrate 5.1 to 60 mm	0.076	0.397	0	0	0	0.1	0	0	0	0	0.2	0	
# pools	0.074	0.215	4	0	2	0	7	5	0	6	2	2	
Nitrate mg/L	0.041	0.296	0.7	1.9	1.1	4.4	0.4	0.7	3.9	0	1	0	
Temperature C	0.022	0.245	18.4	24.9	23.6	22.8	19.9	20.2	22.9	19.3	23.1	24	
Substrate ≤ 0.5 mm	0.018	0.256	0.8	0.9	0.9	0	1	0.2	0.2	0.1	0	1	
Substrate 60.1 - 150 mm	0.000	0.000	0	0	0	0	0	0	0	0	0	0	



**Appendix 2.** Dietary analysis of four fish species at three sites (2011, 2012, and 2341) in the Eastern River system. For each diet category, we present the average proportion (P) of the gut occupied by each diet category, the frequency (F) of guts containing each category, and the relative importance (*I*) of diet categories. The number of guts examined, *n*, is in parentheses next to the species name for each site.

Appendix 2

Site	2011			2012			2012			2012			2012			2012			2012			2012					
	<i>C. striata</i> (1)			<i>C. striata</i> (8)			<i>O. miloiaticus</i> (4)			<i>T. trichopterus</i> (2)			<i>C. striata</i> (15)			<i>O. miloiaticus</i> (12)			<i>T. trichopterus</i> (7)			<i>R. paviei</i> <sup>a</sup> (14)					
Species	P	F	I	P	F	I	P	F	I	P	F	I	P	F	I	P	F	I	P	F	I	P	F	I	P	F	I
Fish	0	0	0.00	13.75	0.25	4.05	0	0	0.00	15	0.5	8.15	7.667	0.133	1.29	0	0	0.00	5	0.25	1.32	17.5	0.214	6.57	0	0	0.00
Shrimp	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0.125	0.00	0	0	0.00	0	0	0.00
Mollusc	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0.125	0.00	0	0	0.00	0	0	0.00
Insect Larvae	12	1	12.00	1.625	0.375	0.72	3.5	1	3.52	6.5	1	7.07	0.833	0.2	0.21	3.417	0.5	1.80	5.857	0.875	5.40	0.714	0.071	0.09	0	0	0.00
Adult Insects	48	1	48.00	23.25	1	27.40	32.25	1	32.43	13.5	1	14.67	59.9	1	75.35	49.42	1	52.21	25	1	26.33	0	0	0.00	0	0	0.00
Oligochaetes	25	1	25.00	43.5	1	51.27	36.75	1	36.96	35	1	38.04	24.13	0.6	18.21	33.5	1	35.40	45.86	1	48.29	63.93	0.786	88.02	0	0	0.00
Macrophytes	0	0	0.00	4.375	0.125	0.64	8.75	1	8.80	7.5	1	8.15	1.833	0.2	0.46	2.5	0.5	1.32	2.857	0.5	1.50	0	0	0.00	0	0	0.00
Sponges	0	0	0.00	0	0	0.00	0.75	0.25	0.19	1	0.5	0.54	0	0	0.00	0.417	0.25	0.11	0.143	0.25	0.04	0	0	0.00	0	0	0.00
Phytoplankton	1	1	1.00	3	1	3.54	6	1	6.03	5	1	5.43	1.4	0.933	1.64	5.333	1	5.64	8.143	1	8.57	4.214	0.643	4.75	0	0	0.00
Zooplankton	14	1	14.00	10.5	1	12.38	12	1	12.07	16.5	1	17.93	4.233	0.533	2.84	5	0.667	3.52	9.286	0.875	8.56	2.286	0.143	0.57	0	0	0.00

<sup>a</sup> Gut content analysis data were only available for this site; therefore, *R. paviei* data were not included in Figure 3.

**Appendix 3.** Spatial survey of fish density using the maximum likelihood estimates (MLE) for sampled sites. Below the site name, the number of passes made ( $k$ ) and the area sampled ( $m^2$ ) are given in parentheses. Probability of capture ( $p$ ), the number of individuals captured ( $n$ ), the MLE of  $n$  ( $\hat{n}$ ), and their standard deviations are provided. For rare species, the MLE is not able to provide SD values for  $\hat{p}$  and  $\hat{n}$ .

### Appendix 3

Site (k,m <sup>2</sup> )	2011 ●			2012 ●			2013 ○			2123 ○						
	n	ñ (sd)	p̂ (sd)	Fish 100 <sup>-2</sup>	n	ñ (sd)	p̂ (sd)	Fish 100 <sup>-2</sup>	n	ñ (sd)	p̂ (sd)	Fish 100 <sup>-2</sup>	n	ñ (sd)	p̂ (sd)	Fish 100 <sup>-2</sup>
A.mas																
A.tes								46	46.0 (0.94)	0.60 (0.07)	52.57	11	11.0	0.65	12.79	
H.nem								20	21.44 (2.48)	0.38 (0.11)	24.50	1	1.0	0.50	1.16	
L.sia																
M.mys								1	1.0	0.99	1.14					
T.acu																
C.lim																
C.str	1	1.0	0.99	1.72	8	8.0	0.80	8.02	15	15.0	0.71	17.14	19	19.0 (1.61)	0.68 (0.18)	22.09
O.nil*	11	11.0	0.99	18.93	82	82 (0.90)	0.82 (0.04)	82.16								
C.bat								1	1.0	0.99	1.14	1	1.0	0.50	1.16	
C.lau																
E.met								86	100.06 (8.23)	0.32 (0.06)	98.29	6	6.0 (2.52)	0.5 (0.44)	6.98	
H.mol*	1	1.0	0.33	1.72												
O.has								1	1.0	0.99	1.14					
L.lep								1	1.0	0.50	1.14					
R.bor																
R.pav	37	37 (1.08)	0.71 (0.09)	63.68				35	35.0 (1.10)	0.64 (0.09)	40.00					
Lobo																
S.bin	1	1.0	0.33	1.72												
S.mar																
R.chi																
D.pus																
M.ery																
M.arm																
G.pet																
G.chu																
T.tri	13	13.0	0.87	22.38	4	4.0	0.67	4.01	63	64.45 (1.87)	0.50 (0.06)	73.66	41	44.33 (3.42)	0.46 (0.09)	51.54
T.vit	5	5.0	0.56	8.61	4	4.0	0.99	4.01	5	5.0	0.56	5.71	4	4.0	0.57	4.65
P.ret*					3	3.0	0.60	3.00								
O.bim									1	1.0	0.33	1.14				
M.alb	1	1.0	0.99	1.72												

**Appendix 3 continued**

Site (k,m <sup>2</sup> )	2231 ○ (4, 68.8)			2232 ○ (4, 61.3)			2233 ○ (3, 195)					
Species	n	$\hat{n}$ (sd)	$\hat{p}$ (sd)	Fish 100 <sup>-2</sup>	n	$\hat{n}$ (sd)	$\hat{p}$ (sd)	Fish 100 <sup>-2</sup>	n	$\hat{n}$ (sd)	$\hat{p}$ (sd)	Fish 100 <sup>-2</sup>
A.mas												
A.tes												
H.nem					3	3.0	0.99	4.89				
L.sia												
M.mys	21	21.0 (1.09)	0.62 (0.12)	30.52								
T.acu												
C.lim					16	16.0	0.80	26.10				
C.str	3	3.0	0.99	4.36	9	9.0	0.69	14.68	6	6.0	0.75	3.08
O.nil*												
C.bat	1	1.0	0.50	1.45	2	2.0	0.99	3.26				
C.lau												
E.met	52	52.0 (1.08)	0.76 (0.07)	75.58					24	24 (2.95)	0.83 (0.26)	12.31
H.mol*												
O.has												
L.lep	1	1.0	0.99	1.45								
R.bor												
R.pav					30	30.0	0.88	48.94				
Lobo												
S.bin					18	18.0	0.82	29.36				
S.mar												
R.chi												
D.pus					1	1.0	0.50	1.63				
M.ery												
M.arm												
G.pet												
G.chu												
T.tri	71	72.69 (2.00)	0.58 (0.06)	105.66	4	4.0	0.50	6.53				
T.vit	9	9.0	0.69	13.08								
P.ret*												
O.bim	2	2.0	0.67	2.91								
M.alb	1	1.0	0.99	1.45					4	4.01 (1.88)	0.50 (0.39)	2.06

Appendix 3 continued

Site (k,m <sup>2</sup> )	2341 ● (4, 156)				2342 ○ (2, 420)				2343 ○ (3, 145)			
Species	n	$\hat{n}$ (sd)	$\hat{p}$ (sd)	Fish 100 <sup>-2</sup>	n	$\hat{n}$ (sd)	$\hat{p}$ (sd)	Fish 100 <sup>-2</sup>	n	$\hat{n}$ (sd)	$\hat{p}$ (sd)	Fish 100 <sup>-2</sup>
A.mas					1	1.0	0.99	0.24				
A.tes	1	1.0	0.33	0.64								
H.nem					8	8.0	0.80	1.90				
L.sia					4	4.0 (3.33)	0.67 (0.76)	0.95				
M.mys												
T.acu					1	1.0	0.99	0.24				
C.lim					2	2.0	0.67	0.48	5	5.0	0.99	3.45
C.str	17	17.12 (1.22)	0.56 (0.13)	10.98					8	8.0	0.99	5.52
O.nil*	183	183.0 (1.42)	0.70 (0.03)	117.95								
C.bat	1	1.0	0.50	0.64					3	3.0	0.99	2.07
C.lau					1	1.0	0.50	0.24				
E.met	63	65.22 (2.36)	0.55 (0.07)	40.38								
H.mol*												
O.has					2	2.0	0.67	0.48				
L.lep					1	1.0	0.99	0.24				
R.bor												
R.pav					4	4.01 (1.88)	0.50 (0.39)	0.95	3	3.0	0.75	2.07
Lobo					7	7.0	0.88	1.67				
S.bin					4	4.0 (3.33)	0.67 (0.76)	0.95	4	4.0	0.80	2.76
S.mar					2	2.0	0.99	0.48				
R.chi					2	2.0	0.67	0.48				
D.pus												
M.ery					1	1.0	0.50	0.24				
M.arm					1	1.0	0.50	0.24				
G.pet					2	2.0	0.99	0.48				
G.chu					2	2.0	0.99	0.48				
T.tri	45	52.11 (6.02)	0.38 (0.09)	33.41	3	3.0	0.75	0.71	1	1.0	0.50	0.69
T.vit	5	5.0	0.50	3.21								
P.ret*												
O.bim												
M.alb					1	1.0	0.50	0.24	10	35.78 (57.65)	0.15 (0.28)	24.67

## **Chapter 2**

**Resource competition and its consequences for fish growth: informing ecological risk  
assessment of non-native *Oreochromis niloticus* in Thailand**

## Introduction

Non-native fishes are commonly thought to adversely change native fish assemblages, both through their ability to outcompete native species for shared resources and through direct predation on native species. Fish routinely escape from aquaculture operations and as this industry expands, the potential ecological effects of escapees on aquatic biodiversity are likely to increase in frequency and importance. The majority of research on the ecological effects of non-native aquaculture escapees and intentional fish introductions concern salmonids in well-studied temperate ecosystems (Pascual 2002). Comparatively little is known about the ecology and biology of both native and introduced fishes in the tropical ecosystems of Asia (Dudgeon 2003), where a diverse array of native and non-native species are cultured with little or no confinement, fish biodiversity is high, and freshwater species hold cultural, religious, economic and dietary value (Coates 2002).

*Oreochromis niloticus* (Nile tilapia) was introduced after World War II to increase food production and is now commonly cultured throughout the region, and feral populations are now common. Speculation on the effect(s) of feral fish on native species ranges from no effect to the decline and extirpation of native species (De Silva et al. 2004). Like other successful freshwater invaders, *O. niloticus* possess a wider range of environmental and habitat tolerances than native species (e.g. Fausch and White 1981; Philippart and Ruwet 1982; Waters 1983; Flecker and Townsend 1994; Sorensen et al.



1995; Townsend 1996; Essington et al. 1998; Waters 1999; Biggs et al. 2000; Grant et al. 2002). In its native range, *O. niloticus* tolerate a more narrow range of environmental and habitat variables than in their naturalized range, within which there is considerable variation (Lever 1996). Such niche expansion and flexibility underscores the need for site-specific research to inform ecological risk assessment (ERA) to determine how *O. niloticus* may affect native fish assemblages.

Competition is one of the most important mechanisms for individuals to affect one another, directly or indirectly (level V), which can lead to an adverse change in the fish assemblage and a loss of native biodiversity (level VI). Risks to the assemblage include any adverse effects on native species or ecological processes that affect the assemblage. The potential for competition between feral fish and conspecifics, closely related species, and heterospecifics is significant if there is high dietary and habitat overlap (McGinnity et al. 1997; Fleming et al. 2000; Volpe et al. 2000; Pascual et al. 2002; McGinnity et al. 2003; Baxter et al. 2004). In small streams in the Eastern River system in southeastern Thailand, feral *O. niloticus* are abundant and have high habitat and dietary overlap with native species (levels I – III; Chapter 1); *O. niloticus* have been associated with the decline of a native species, *Trichogaster trichopterus* (Bang Prah Fishery Manager, Thai Department of Fisheries, personal communication). *O. Niloticus* and *T. trichopterus* also have similar ecological characteristics—males establish and aggressively defend territories and parental care is provided. Competition between

animals, through their behavioral interactions and resource use, can negatively affect growth and survival of either or both animals involved in the interaction. Competition is thus a potential mechanism for *O. niloticus* to negatively affect *T. trichopterus* due to their reliance on shared resources (level III).

Exploitative competition may not be as important as interference competition due to these species' generalized diets (Chapter 1; Senanan, Burapha University, Thailand, unpublished data). In addition, both species are aggressive and territorial and could therefore interfere with each other's ability to efficiently obtain resources. Aggression is an energetically costly behavior in species which exhibit territoriality and social dominance hierarchies. Intraspecific competition between feral salmon from farms and native wild salmon in temperate freshwater ecosystems has been shown to result in increased stress, decreased growth, and displacement; shifting native salmonids to less desirable habitats in the presence of feral farmed salmon (McGinnity et al. 1997; 2003; Fleming et al. 2000). Virtually nothing is known about interference competition between feral *O. niloticus* farm escapees and native fish in tropical freshwater ecosystems.

Feral *O. niloticus* and native species support important capture fisheries in Thailand. Growth-enhanced selectively bred *O. niloticus* were introduced to Thailand for farming in 1994 (Eknath et al. 1998) and have likely become feral as well. Selectively bred salmonids have been shown to grow faster, be more aggressive, and consequently

negatively affect wild conspecifics and native species in their naturalized environment (Fleming and Einum 1997; Fleming et al. 2002). Selectively bred *O. niloticus* that become feral may have similar negative effects on conspecifics and native species in Thailand. For this reason, we examined the effects of both selectively bred and feral *O. niloticus* on native species.

The aim of this study was to determine whether interspecific competition between *O. niloticus* and *T. trichopterus* is important, via behavioral experiments (level IV), and to measure its effect on growth (level V). In the parlance of an ERA fault tree, this study focused on informing the assessment of effects (levels IV and V) that are necessary for a non-native fish species to cause an adverse change in the native fish assemblage (the ecological response). The specific objective of this study was to compare the effects of two factors, *O. niloticus*-type (both size and genetic background) and fish density, on the growth response of *O. niloticus* and *T. trichopterus*. We used two methodologies under the same experimental design: one to study the mechanism of impact (competitive behavior, level IV), and another to measure the response (change in growth over time, level V).

## **Materials and Methods**

To increase the ecological relevancy of our findings, we incorporated several key environmental parameters into the experimental environment and research animals were similar to those found in syntopic populations in our study area.

## Research animals

We selected species with genetic backgrounds, at life stages, and at relative densities that were consistent with those found in our study area in Thailand. Syntopic populations consist of immature *O. niloticus* and *T. trichopterus* adults at varying stages of sexual maturity. There are several varieties of *O. niloticus* used for fish farming in our study area, including farmer- and selectively-bred varieties. We collected feral *O. niloticus* juveniles (hereafter, the feral line) from the Bang Prah reservoir which is near our study area (Chapter 1) for use in experiments. These fish were likely hatched in the wild given their size at capture (total length;  $L_T < 3\text{cm}$ ). The feral line was transported and reared to adulthood in our laboratory, and their first generation offspring were incorporated into experiments. The selectively bred line that we used in experiments is available for aquaculture in Thailand (Dey 2000). The WorldFish Center in Malaysia sent us juveniles to our laboratory where they were acclimated and incorporated into the experiment. The *T. trichopterus* were a cultured strain obtained from a US supplier.

All fish within an experimental tank were size matched within species using relative sizes (i.e.  $L_T$ ) from the field. Two sizes of the feral line (hereafter  $ON_{FE}$  and  $ON_{fe}$ ) were used in experimental trials;  $ON_{FE}$  were size matched to *T. trichopterus* (hereafter TT); and  $ON_{fe}$  were within the size range of *O. niloticus* captured at field sites. The  $ON_{FE}$  were size matched to TT to test for an effect of different initial sizes on dominance status and growth. The selectively bred line (hereafter  $ON_{sb}$ ) was also sized according to the sizes of *O. niloticus* captured in the field. See Figure 1 for a graphical description of

fish types captured in the field and used in the laboratory. In the field, *T. trichopterus*  $L_T$  ranged from 2.5 – 9.0 with  $L_T = 6.04$ , and feral *O. niloticus*  $L_T$  ranged from 1.0 – 10.0 with  $L_T = 2.97$ . The initial  $L_T$  for  $ON_{FE}$ ,  $ON_{fe}$ , and  $ON_{sb}$  used in experiments was 5.93, 3.81, and 3.98 respectively. Relative fish densities in all treatments approximated natural densities. *T. trichopterus* field densities were estimated at 0.01 – 1.06/m<sup>2</sup> with an average of 0.33/m<sup>2</sup>, and feral *O. niloticus* densities were estimated at 0.19 – 1.17/m<sup>2</sup> with an average of 0.73/m<sup>2</sup> (Chapter 1).

### Experiment environment

Experimental conditions were designed to simulate characteristics of the natural environment while allowing for easy observation of fish behavior. Experiments were conducted in 113.56 L glass aquaria with a shelter (5.08 cm diameter PVC pipe), a feeding ring suspended at a point furthest from the shelter, and 0.1 - 0.5 cm diameter gravel substrate, which was similar to that found in the field (Figure 2). All fish, in holding and experimental tanks, were supplied by a continuous flow of water from a 15,141 L recirculating water system supplied by well water. Water ( $\bar{T} = 28.8 \pm 1.75^\circ\text{C}$ ) entered tanks through three flexible silicone tubes lying on the gravel substrate and exited through a 2.54 cm diameter hole drilled in the upper left corner of the tank throughout the study period. The artificial lighting system was set to mimic ambient conditions, providing 30 minutes of sunrise and sunset low-light conditions and 12 hours of full daylight.

Fish were fed to excess with a crumbled 35% protein diet (manufactured by Silver Cup Inc.) via automatic feeders that dispensed food almost continuously throughout the day-light period. Food was introduced through the feeding ring in excess (2.2 g /day) and continuously from day 2 through day 29 of the 31 day experiment. Food dropped into the ring, floated out of the ring on the surface of the water, then sank to the substrate. Tank ration was the same regardless of fish density, species composition, or *O. niloticus*-type. Although dispensing of food was spatially predictable, and not necessarily representative of the natural environment, it was designed to allow for identification of social hierarchies (Sloman et al. 2000).

#### Experimental design

We designed a laboratory experiment to examine both the mechanism for and the outcome of competition between individuals and species with different densities and sizes (Figure 3). Additive density schemes were used to examine evidence for competition between species while competition was confounded with a change in total fish density (Fausch 1988; 1998). We utilized three additive density schemes, using equal (AdE<sub>1</sub> and AdE<sub>2</sub>) and unequal (AdU) numbers of each species. The AdU density scheme used a species ratio reflective of relative abundance of each species in the field. The AdE<sub>1</sub> and AdE<sub>2</sub> density schemes used equal starting densities that did not confound treatment effects (Underwood 1986; Ruetz et al. 2003). In addition, we employed one substitutive scheme (Sub) using equal numbers of each species. This density scheme was used to examine the relative strength of intra- versus inter-specific competition

(Underwood 1986; Fausch 1988; 1998; Weber and Fausch 2003; 2005). Employing all density schemes simultaneously allows for the examination of the magnitude of interspecific competition relative to intraspecific competition, and the importance of adding a second species relative to increases in density of the same species on fish growth (Fausch 1998; Underwood 1986; Zimmerman and Vondracek 2006).

We used an incomplete and unbalanced randomized block experimental design with 22 treatments to examine how treatments affected fish growth. The experimental unit was tank for each species. The design was unbalanced because there were more treatment levels for *O. niloticus* than for *T. trichopterus*. A complete design would have included four full replicates of all treatments. It was incomplete because only one full replicate of each single species *T. trichopterus* (hereafter TT) treatment level was performed and these treatments were assigned in a completely randomized design across all blocks. *O. niloticus*-type (hereafter ON<sub>x</sub>), had three levels (ON<sub>FE</sub>, ON<sub>fe</sub>, and ON<sub>sb</sub>), which were assigned in a randomized complete block design due to the expected growth differences between levels. The experiment was blocked on units of time (n = 12). The treatment, fish density, had 13 levels for TT (single species: 3TT, 2TT, 1TT, 4TT; and mixed species 3TT+3ON<sub>x</sub>, 2TT+2ON<sub>x</sub>, 1TT+3ON<sub>x</sub>), and 18 levels for ON<sub>x</sub> (single species: 3ON<sub>x</sub>, 2ON<sub>x</sub>, 4ON<sub>x</sub>; and mixed species: 3TT+3ON<sub>x</sub>, 2TT+2ON<sub>x</sub>, 1TT+3ON<sub>x</sub>) (Figure 3). ON<sub>x</sub> was randomly applied to blocks within replicates, treatments were randomly assigned to tanks within blocks, and fish were randomly assigned to tanks. Four replicates of each treatment were conducted for a total of 12 blocks. All replicates of

treatments were randomly assigned to tanks across blocks. Also, duplicated treatments (i.e. 3ON<sub>x</sub> for both AdE<sub>1</sub> and AdU, and 2TT+2ON<sub>x</sub> for both AdE<sub>2</sub> and Sub) were not conducted.

#### Fish measurement and identification

Fish were measured and their  $L_T$  and weight (g) were recorded on day 1 and 31 of the experiment. Individuals were anaesthetized with a solution of Tricaine Methanesulfonate (2 mg/L) and given fin clips to enable unique identification of fish within tanks (Stuart 1958). No adverse anesthetic or fin clip effects were observed on fish behavior, health, or growth. Fish were then allocated to groups of size-matched fish (maximum size difference  $\pm$  0.01 cm) to control for size effects on dominance (Huntingford et al. 1990). On days 10 and 21 fish were simultaneously captured and given fresh fin clips to maintain accurate identification. This experiment was carried out under the approval of the University of Minnesota's Institutional Animal Care and Use Committee.

#### Behavior

Forty-five behavioral observations were made over the 31 day experimental period for each treatment tank. Experiments were conducted in a controlled environment. Opaque panels kept fish from reacting to fish in other tanks, the researcher, reflected light, and other distracting stimuli. We recorded fish behavior using high resolution 550 TVL, 2.6 – 6 mm lens Nuvico digital cameras with a Dell Geovision 240 FPS digital video recorder.



Three 30-minute observation recordings were made daily (800h, 1200h, and 1900h) for five consecutive days beginning on day three of the experiment. Six point observations (1 minute duration) were made at equal intervals throughout each 30-minute observation. In addition, each tank was visually observed daily. Presence of nests, excess or fouled food, fish health, and activities of individuals were recorded.

In each point observation, we monitored behavior and physical position in the tank, relative to the food source, for each fish in six behavioral classes: (1) feeding, (2) relative feeding position, (3) social interactions, (4) concealment, (5) physical display, and (6) position (Table 1). Food was automatically introduced to each tank throughout all observation periods. We recorded where fish fed in the water column (e.g. at the surface or from the substrate) (class 1), and their feeding position relative to the food source (class 2; Figure 2). Social interactions were the most complex type of data to score. *O. niloticus* are social animals (Volpe et al. 2003). Their interactions were fast and often involved multiple individuals in a cascade of interactions. Chasing, frontal contests, biting, mouth fighting, territorial defense, nest site defensive actions and breeding displays were recorded as social interactions (class 3). We also scored fish for being the object of an aggressive social interaction (e.g. biting and chasing), and for acting in a submissive-manner as a result of an aggressive interaction (e.g. retreating from a territory when contested). Social interactions were viewed in slow motion using digital recordings to correctly record each behavior. We had three measures of concealment (class 4): whether or not a fish swam actively in the water column, used

the shelter as a refuge, or maintained a stationary position. Fish presented physical coloration displays, which have been associated with dominance status (Beeching 1995; Barcellos et al. 1999; Volpato et al. 2003). We recorded the presence of such displays as well as signs of injury and all mortalities that occurred during the experimental period. Fish were also scored according to their position in the tank (class 6), i.e. the quadrants that they most often occupied (Figure 2).

We developed a weighted scoring system (Table 1) to assess the relative social status of individuals in each treatment based on a similar system developed by Metcalfe et al. (1989) and utilized in several other studies examining how fish behavior results in growth and dominance (e.g. Johnsson et al. 1996; Sloman et al. 2000; 2001; Harwood et al. 2003). This scoring system is weighted for behaviors and positions that are most associated with dominance status (Sloman et al. 2000). A fish received a point (positive value) for an aggressive behavior, no points (zero value) for being the subject of an aggressive behavior, and they lost points (negative value) if they then behaved in a submissive manner (e.g. retreating from a territory, hiding in the shelter). Feeding scores were identified with the feeding location (surface, within the feeding ring, and from the substrate). Points were assigned for the position that a fish held based on relative distance from the food source. Fish that held positions closest to the feeding ring were given the highest score. The fish next closest to the ring received the next highest score and so on for the remaining individuals. See Table 1 for point values for each behavior and position in the scoring system.

We summed the point values over all behavior classes to create a dominance score for each individual. We used the relative dominance scores within each treatment tank to rank fish in order of decreasing dominance score: the fish with the highest score was considered dominant and assigned rank 1; the fish with the second highest score was subdominant and assigned rank 2. The remaining fish were assigned descending ranks according to their individual status in the tank.

### Growth

We measured the growth response as the change in growth over time. Fish were measured ( $L_T$  and mass) on day 1 and day 31 of the experiment, and measurements were made to the nearest 0.01 cm and 0.001 g. We measured the growth response as the change in growth over time (31 days) using Ricker's (1979) equation for instantaneous rate of growth ( $G$ ) for an interval of time  $t_f - t_i$ ,

$$G_W = 100 * (\ln w_f - \ln w_i) / t_f - t_i$$

with  $w_f$  as the final weight and  $w_i$  as the initial. A similar equation was used to calculate  $G$  for length ( $G_L$ ).

Treatments were designed to compare the effects of increasing fish density and species composition (single versus mixed species treatments) on the growth of *T. trichopterus* and each level of  $ON_x$ . For example, we could compare the growth of *T. trichopterus* alone versus their growth with  $ON_{FE}$ ,  $ON_{fe}$ , or  $ON_{sb}$  for each density scheme. A single ANOVA model was fit to the data by species. We examined differences in the

growth response for treatments in each density scheme using the ANOVAs and the Tukey-Kramer multiple comparison test for unequal cell sizes (JMP; SAS Institute 2008). All significance thresholds were based on  $\alpha = 0.05$ .

### Behavior and Growth

We examined the relationship between fish growth ( $G$ ) and dominance rank (1 – 6) in each treatment for each fish-type (TT and  $ON_x$ ) using ANOVAs and Tukey-Kramer multiple comparison tests. All significance thresholds were based on  $\alpha = 0.05$ . We also used overlay plots to visualize the relationship between dominance rank and growth for TT and  $ON_x$ .

## **Results**

### Behavior

Dominance hierarchies appeared among individuals in all treatment tanks. Position and social interactions were strong indicators of dominance status. All fish ate throughout all observations, as food was abundant in tanks, and all fish grew over the course of the experiment. In spite of strong agonistic interactions, there were few mortalities. The most dominant (rank 1) fish often held positions and defended territory close to or directly under the feeding ring. They readily chased, bit, and otherwise engaged aggressively with individuals attempting to enter their territory; and they most often held their position when contested. The subdominant (rank 2) often held positions in quadrants at the next closest position relative to the dominant fish. They often

attempted to enter the territory of the dominant fish, which chased and bit this subdominant individual until they retreated from the territory. When the dominant fish vacated its position, the subdominant often entered the quadrant immediately and took position. Upon return of the dominant fish, the subdominant would either immediately retreat from the territory or contest the dominant fish. In addition to attempting to gain access to the dominant fish's resources, the subdominant fish often directed aggressive behaviors toward lower ranked fish (ranks 3, 4, 5 and 6), regardless of their actions or position in the tank.

The behavior of subordinate fish (ranks  $\geq 3$ ) varied depending on fish density in their treatment tank and the species occupying the dominant position (under the feeding ring). In single species treatments with more than two individuals, subordinates were infrequently aggressive towards more dominant fish. Instead, their social interactions usually involved submissive behaviors in response to aggressive actions directed toward them. The lowest ranking fish held positions furthest from the food source, and they rarely attracted attention from higher ranking fish. *O. niloticus* individuals held rank 1 in 86.1% of mixed species treatments, and all *T. trichopterus* were entirely subordinate to all *O. niloticus* in 67.7% of all treatment tanks. Within species in mixed species treatments, subordinate fish (rank  $\geq 3$ ) rarely exhibited dominant-type behaviors towards fish ranked 1 and 2; however, in the five tanks that *T. trichopterus* held rank 1 (AdU n = 3 and Sub n = 2) the subordinate *O. niloticus* often acted in a subdominant-manner, but contested the *T. trichopterus* and attempted to

gain access to its position in the tank. It was rare for *O. niloticus* to occupy subordinate positions in mixed species treatments: in the AdE<sub>1</sub> mixed species treatment tanks, *O. niloticus* occupied rank 4 once and never occupied ranks 5 or 6; and in the Sub treatment tanks, *O. niloticus* occupied rank 4, the lowest available in this treatment, only twice (Appendix 1).

Fish appeared to readily assume positions within each tank and maintain them throughout the experiment. The primary position occupied by each species and individual was established between 72 hours and 7 days, respectively, after the initiation of the experiment. We observed coups in 13% of treatment tanks, resulting in two fish exchanging their relative positions. Coups always involved competition for the position closest to the feeding ring. Three coups were staged in mixed species tanks (AdE<sub>1</sub> and AdU treatments); and, in one instance, a *T. trichopterus* individual successfully replaced an *O. niloticus* individual in the position directly under the ring. In another case, *O. niloticus* replaced *T. trichopterus* in this position. All other coups were intraspecific.

### Growth

We fit the growth ANOVA models using the  $\bar{G}_L$  and  $\bar{G}_W$  for each species in each treatment tank. We focus below on growth results in terms of  $\bar{G}_L$  because both growth measures ( $\bar{G}_L$  and  $\bar{G}_W$ ) had similar responses unless otherwise noted. Condition factor

was not used as a growth measure due to the varying states of sexual maturity present within treatment tanks and among *O. niloticus*-types used in the experiment.

#### *T. trichopterus*

Growth of *T. trichopterus* was significantly affected by treatments, which explained 68.9% of the variation in the difference in growth among treatments ( $\bar{G}_L F_{12} = 5.2391$ ,  $p < 0.0001$ ; Table 2). Overall, *T. trichopterus* growth was not significantly affected by density treatment or species composition (i.e. single versus mixed species treatments) although small differences did occur (Appendix 2 and 3). In mixed species treatments, *O. niloticus*-type significantly affected the growth of *T. trichopterus* ( $\bar{G}_L F_2 = 7.1775$ ,  $p = 0.0025$ ). Tukey-Kramer multiple comparison tests of the means indicated that *T. trichopterus* grew the least in mixed species treatments with  $ON_{sb}$  (Figure 4a and Appendix 2). It is interesting to note that *T. trichopterus* growth declined as fish density increased in mixed species treatments, with the exception of the AdU treatment with  $ON_{fe}$ , in which *T. trichopterus* had the highest growth response across all treatments and the lowest response when combined with  $ON_{sb}$  (Figure 4a). In addition, *T. trichopterus* had slightly lower growth when combined with  $ON_{sb}$  in the Sub and AdE<sub>1</sub> mixed species treatments, although the decline was not as extreme and neither was significant.

#### *O. niloticus*

*O. niloticus*-type significantly affected the growth of *O. niloticus* and explained 58.1% of the variation in the difference in growth among treatments ( $\bar{G}_L F_{17} = 8.8165$ ,  $p <$

0.0001; Table 2). Fish density and the presence of *T. trichopterus* alone did not result in significant differences in growth; however, it is interesting to note that  $ON_{fe}$  had higher growth in mixed species treatments than in single species treatments (Figure 4b). In addition, the  $ON_{sb}$  grew more over all treatments compared to the feral-line (Figure 4b). A detailed description of the growth of each  $ON_x$  level in single and mixed species treatments is presented in Appendix 2 and Appendix 3.

### Behavior and Growth

There was a strong association between the dominance rankings assigned by the scoring system and the outcomes of social interactions on fish growth for both species. In spite of size-matching all fish by species within treatment tanks at the initiation of the experiment, growth differences soon became apparent among individuals and these differences were associated with dominance status. The association between growth and dominance status was strongest for *T. trichopterus*. Overall, their growth differed significantly by social rank ( $F_{5,102} = 4.1775$ ,  $p = 0.0017$ ). Rank 1 *T. trichopterus* grew significantly more ( $G_L$  and  $G_W$ ) than subordinates (Table 3), and overall *T. trichopterus* categorized as rank 1, 2 and 3 grew significantly more than *T. trichopterus* in rank 4 – 6, as indicated by Tukey-Kramer multiple comparison tests. The association between social rank and growth was also significant for  $G_W$  in *O. niloticus* ( $\bar{G}_W F_{3,200} = 4.6213$ ,  $p = 0.0038$ ), but not for  $G_L$ . The growth of  $ON_{FE}$  was significantly affected by dominance rank ( $\bar{G}_L F_{3,64} = 6.1420$ ,  $p = 0.0010$ ); rank 1  $ON_{FE}$  grew significantly more than lower ranking fish, as indicated by Tukey-Kramer multiple comparison tests. This relationship also



occurred for  $ON_{sb}$  and  $ON_{fe}$  although growth differences by social rank were not significant. See Appendix 1 for a graphical depiction of growth by dominance rank for fish in each treatment by fish-type.

## **Discussion**

This research was organized around an ecological risk model, consisting of a fault tree of events necessary for a non-native species (the ecological stressor) to cause an adverse change in native fish assemblages (the ecological response). Changes that are most often associated with the establishment of non-native fishes are catastrophic and include substantial changes in the range or abundance of a native species and in some cases, their extirpation (level VI). Less obvious but more measurable consequences include increased competition for food and habitat (level IV) that can lead to changes in growth of individuals (level V) (Ricklefs and Miller 2000). Such ecological effects, alone or in combination with other ecological stressors, e.g. habitat degradation, could lead to more substantial adverse effects (level VI) for fish assemblages. Our study documented the mechanism and outcome of interference competition between non-native *O. niloticus* and a native species, *T. trichopterus*.

We examined competitive interactions within and between species in both single and mixed species treatments and found a strong association between dominance rank and growth for each species. Dominance hierarchies were readily established in all treatment tanks. Overall, the dominant fish had primary access to the food resource.

The subdominant fish often attempted to gain access to this resource and was aggressive to subordinates, which often held positions furthest from the feeding ring. *T. trichopterus* and *O. niloticus* with low social status (rank  $\geq 3$ ) grew significantly less than subdominant and dominant individuals, which often had greater access to profitable resources in the treatment tank. This finding indicates that it is most beneficial to be a dominant fish in the hierarchy with regard to resource availability and growth rate, which is in agreement with other studies in laboratory (Abbot and Dill 1989; Pottinger and Pickering 1992), semi-naturalized (Li and Brocksen 1977; Fausch 1984), and natural systems (Höjesjö et al. 2002).

The consequences of interference competition, as measured by behavior and dominance ranking, appear to be especially relevant for *T. trichopterus*. In mixed species treatment tanks, *O. niloticus* were the dominant fish (rank 1) in 86.1% of all treatment tanks, and all *T. trichopterus* individuals were subordinate to *O. niloticus* in 67.7% of treatment tanks. *T. trichopterus* grew significantly less in some mixed species treatments relative to when *O. niloticus* were absent. Although interactions appeared severe in treatment tanks, few mortalities or injuries were observed, and the shelter was not often used as a refuge by either species. These results suggest the potential for *O. niloticus* to strongly affect *T. trichopterus* populations (level V) through interference competition.

Our results show evidence of interference competition occurring within and among species, with *O. niloticus* acting as the superior competitor in most instances when they co-existed with *T. trichopterus*. The outcome of interference competition was measured as growth differences between single and mixed species treatments under four density schemes. These schemes were employed simultaneously to enable the examination of the relative effects of increasing fish density versus substituting equal numbers of an additional species on fish growth rates. Our experiment showed no significant differences in the growth of each type of *O. niloticus* in single or mixed species treatments for any density scheme, although growth of this species appeared to decline slightly in mixed species treatments relative to single species treatments overall.

*T. trichopterus* growth was significantly higher when grown alone than when  $ON_{sb}$  were present. This result was also reflected in the growth of *T. trichopterus* with  $ON_{FE}$ , although the difference in growth between single and mixed species treatments for this *O. niloticus*-type was not significant. In contrast to these findings, the growth of both *T. trichopterus* and  $ON_{fe}$  increased slightly when grown together in mixed species relative to single species treatments, with the exception of the  $AdE_1$  density scheme for *T. trichopterus*. The sizes of both  $ON_{fe}$  and *T. trichopterus* were matched to the size range of each species captured from syntopic populations in our study area. The  $ON_{sb}$  were also size matched to the *O. niloticus* captured in the field, but  $ON_{sb}$  and *T. trichopterus* did not present a mutual benefit to one another. Rather,  $ON_{sb}$  resulted in a significant decline in *T. trichopterus* growth.

We would only expect to observe interspecific competition under conditions where intraspecific competition occurs due to the increased reliance on shared resources by individuals of the same species (Underwood 1986). *T. trichopterus* in single species treatments at a density of two fish (AdE<sub>2</sub>) exhibited the lowest growth over all single species treatments. Behavioral interactions were often severe in these treatment tanks relative to other treatments, and may likely have resulted in lower growth as a result. Increasing density was found to result in lower growth in all other single species treatments (i.e. growth of 1TT > 3TT > 4TT). Although growth declined as density increased in single species treatments of each species, it was not significant in either species. This might be because *O. niloticus* and *T. trichopterus* densities were not high enough in single species treatments to observe evidence of intraspecific competition.

The differences in relative fish densities among all density schemes were small, and therefore may not have been large enough to detect differences in the interactions among treatments within and among the schemes as well. The addition and substitution of ON<sub>sb</sub> to tanks under the AdE<sub>1</sub>, AdE<sub>2</sub>, and Sub density schemes did result in significantly lower growth in *T. trichopterus*. This species was less able to compete with *O. niloticus* as evidenced by their complete subordinate rank relative to *O. niloticus* in 67.7% of all treatment tanks, and resource use by ON<sub>sb</sub> may have been greater than that of *T. trichopterus*. The effect of ON<sub>sb</sub> on *T. trichopterus* was further evidenced by the fact that *T. trichopterus* was dominant to ON<sub>sb</sub> in only one treatment tank.

Our experiment illustrates the type of field-informed manipulative experiments that risk assessors will need to conduct in order to quantitatively estimate the probability of competitive interactions and the magnitude of these effects on fish assemblages in a site-specific ERA of any introduced fish. Before our study, competition and its effects were largely assumed, but never tested for *O. niloticus*. We found that, although *O. niloticus* individuals were not negatively affected by treatments, the *T. trichopterus* grew significantly less when in the presence of ON<sub>sb</sub> and were subordinate to *O. niloticus* in most treatments. This result could have important implications for the use of growth-enhanced selectively bred varieties of *O. niloticus* in aquaculture especially given ongoing interest and efforts to disseminate selectively bred *O. niloticus* to farmers in the tropics (ADB 2005). More in depth analysis of the frequency and duration of agonistic behavioral interactions between individuals and species when *T. trichopterus* are held with ON<sub>sb</sub>, relative to the feral, could further elucidate this finding.

Similar laboratory studies have shown that the benefits of being a dominant individual in fish species that form social hierarchies can be exaggerated in the laboratory compared to the natural environment (Höjesjö et al. 2002; Harwood et al. 2003). Our experimental environment was informed by our field research (Chapter 1) in an attempt to mitigate this laboratory effect; however, water clarity in the laboratory was higher than that found in the field and this may have implications for the presence and strength of competitive interactions seen in this study. We did, however, observe competitive interactions between individuals when the lights were off in the laboratory,

indicating that the observed competitive interactions were not reliant on the higher visibility of clear-water conditions present in our experiment. This study was designed to determine whether or not interference competition occurs between non-native *O. niloticus* and a native species with high resource overlap. The ability to observe fish and their behavior was thus critical. We have shown that competition does indeed occur when fish sizes and densities are within the range found in the wild. The next step in research aiming to inform environmental risk assessment of *O. niloticus* would be to conduct similar manipulative experiments in a field setting. The natural environment is likely to be more heterogeneous, less spatially restricted, and have decreased water clarity compared to the laboratory environment, which may allow for more diverse behavioral strategies to occur.

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**Table 1.** Scoring system and weighted values used to measure dominance status and assign social rank in experimental treatments. Each fish could receive points in each behavior class during each point observation. The value assigned to each observation score corresponded to the type of interaction. Positive values were assigned to aggressive actions, and zero and negative values were assigned to behaviors that resulted from the aggressive actions of another individual. Higher scores are indicative of more dominant behavior, and fish with the highest cumulative score in each tank was considered to be dominant and assigned rank 1.



**Table 1**

<b>Behavior class</b>	<b>Behavior action and location</b>	<b>Point value</b>
1 Feeding	Eating at ring	1
	Eating at surface	0.5
	Eating from substrate	0.25
	Failure to eat	0
2 Relative feeding position	Closest to food source	5
	Second nearest to food source	4
	Third nearest to food source	3
	Forth nearest to food source	2
	Fifth nearest to food source	1
	Furthest from food source	0
3 Social interaction	Chase initiator	1
	Chase recipient	0
	Contest initiator	1
	Contest recipient	0
	Bite initiator	1
	Bite recipient	0
	Mouth fight initiator	1
	Mouth fight recipient	0
	Territory defense	1
	Territory retreat	-0.5
	Shelter defense	1
	Nest site defense	1
	Nest site occupation	0.5
	Dominate intraspecifically	1
	Dominate interspecifically	2
	Coup d'état winner	1
Coup d'état loser	-0.5	
4 Concealment	Swimming in the water column	1
	Positioned in the shelter	-0.5
	Stationary	0
5 Physical display	Erect fins, pink body coloration, light eyes <sup>α</sup>	2
	Darkened body coloration, dark eyes <sup>α</sup>	1
	No special coloration	0
	Injured	-0.25
	Mort during trial from injuries	-0.5
6 Position	Feeding ring	4
	Feeding ring periphery	2.5
	Quadrant <b>a</b>	3
	Quadrant <b>b</b>	2
	Quadrant <b>e</b>	1.25
	Quadrant <b>f</b>	1
	Quadrant <b>g</b>	0.75
	Quadrant <b>c</b>	0.5
	Quadrant <b>d</b>	0

<sup>α</sup> Eye color not used as an indicator for *T. trichopterus*.

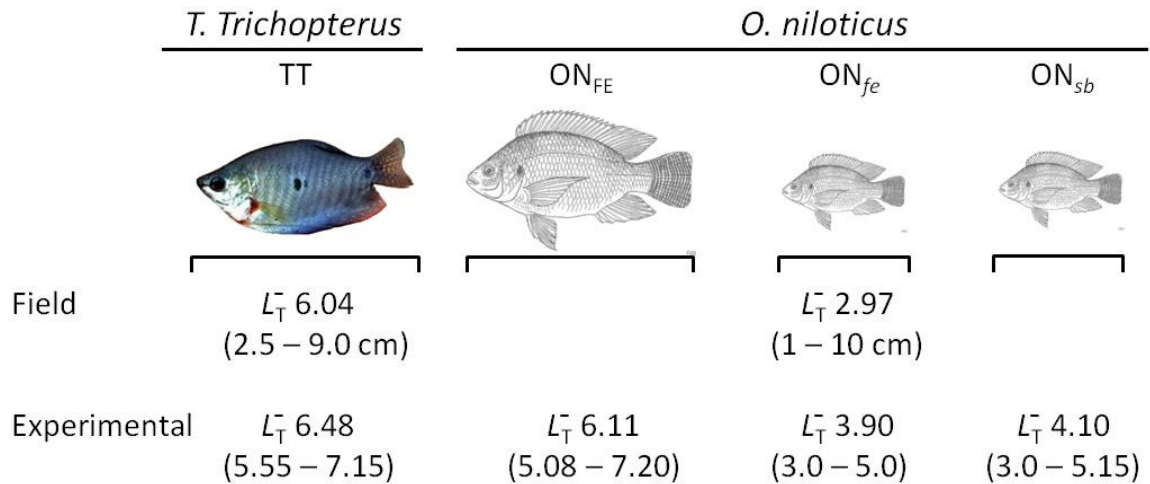
**Table 2.** Analysis of variance (ANOVA) test statistics for the incomplete and unbalanced randomized block design model with treatment as the main factor (13 levels for *T. trichopterus* and 18 levels for *O. niloticus*).

	<i>T. trichopterus</i>				<i>O. niloticus</i>				
	R <sup>2</sup>	F	df	p	<i>G<sub>L</sub></i>	R <sup>2</sup>	F	df	p
<i>G<sub>L</sub></i>	0.68858	5.2391	12	< 0.0001	<i>G<sub>L</sub></i>	0.58091	8.8165	17	< 0.0001
<i>G<sub>W</sub></i>	0.68744	7.0486	12	< 0.0001	<i>G<sub>W</sub></i>	0.75944	5.4483	17	< 0.0001

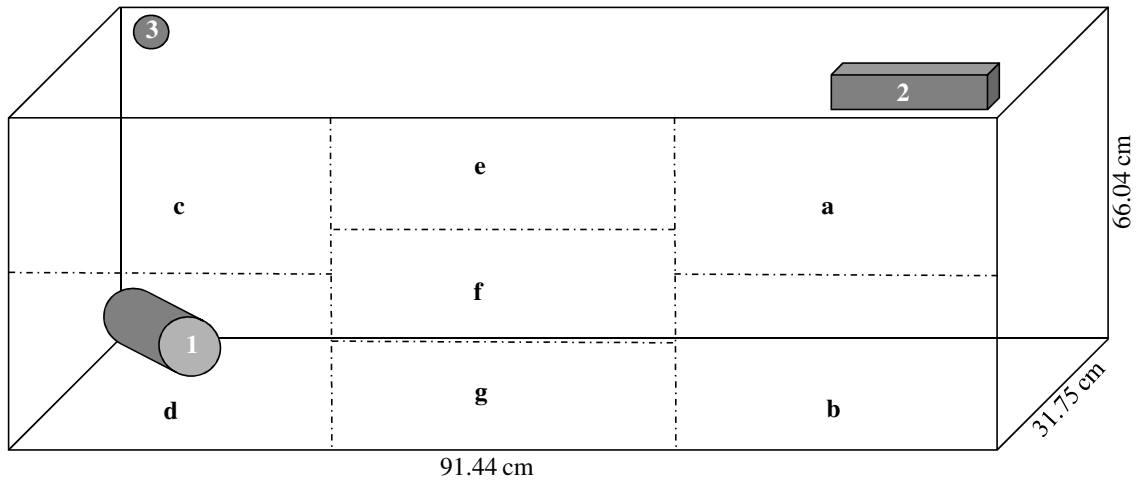
**Table 3.** Analysis of variance (ANOVA) test statistics for the instantaneous growth rate ( $G$ ) for length ( $G_L$ ) and weight ( $G_W$ ) by dominance rank of individuals within each treatment for *T. trichopterus* and *O. niloticus*. The dominant fish in each treatment tank had the highest total score (see Table 1) and was awarded rank 1, the subdominant rank 2, and subordinate fish awarded rank 3 through 6. Levels not connected by the same letter are significantly different as indicated by the Tukey-Kramer test for multiple comparisons.

**Table 3**

$G_L$					$G_W$				
All fish by rank									
$R^2$	F	df	p		$R^2$	F	df	p	
0.2332	18.613	5, 306	< 0.0001		0.2525	20.671	5, 306	< 0.0001	
	Rank	Mean	SE			Rank	Mean	SE	
	1	2.17	0.09	A		1	6.04	0.25	A
	2	1.83	0.09	A B		2	5.22	0.25	B
	3	1.77	0.10	B		3	5.17	0.28	B
	4	1.05	0.13	C		4	3.12	0.35	C
	5	0.49	0.23	C		5	1.65	0.63	C
	6	0.49	0.35	C		6	1.49	0.96	C
Species by rank									
<i>T. trichopterus</i>									
$R^2$	F	df	p		$R^2$	F	df	p	
0.1700	4.1775	5, 102	0.0017		0.2152	5.5927	5, 102	0.0001	
	Rank	Mean	SE			Rank	Mean	SE	
	1	0.86	0.06	A		1	3.00	0.21	A
	2	0.64	0.05	A B		2	2.32	0.17	A B
	3	0.61	0.06	B		3	2.25	0.2	A B
	4	0.60	0.05	B		4	1.97	0.15	B
	5	0.49	0.07	B		5	1.65	0.22	B
	6	0.49	0.10	B		6	1.49	0.34	B
<i>O. niloticus</i>									
$R^2$	F	df	p		$R^2$	F	df	p	
0.0429	2.9862	3, 200	0.0323		0.0648	4.6213	3, 200	0.0038	
	Rank	Mean	SE			Rank	Mean	SE	
	1	2.47	0.08	A		1	7.18	0.24	A
	2	2.21	0.08	A		2	6.27	0.24	B
	3	2.19	0.09	A		3	6.11	0.27	B
	4	2.04	0.18	A		4	5.67	0.53	B
	5	na				5	na		
	6	na				6	na		



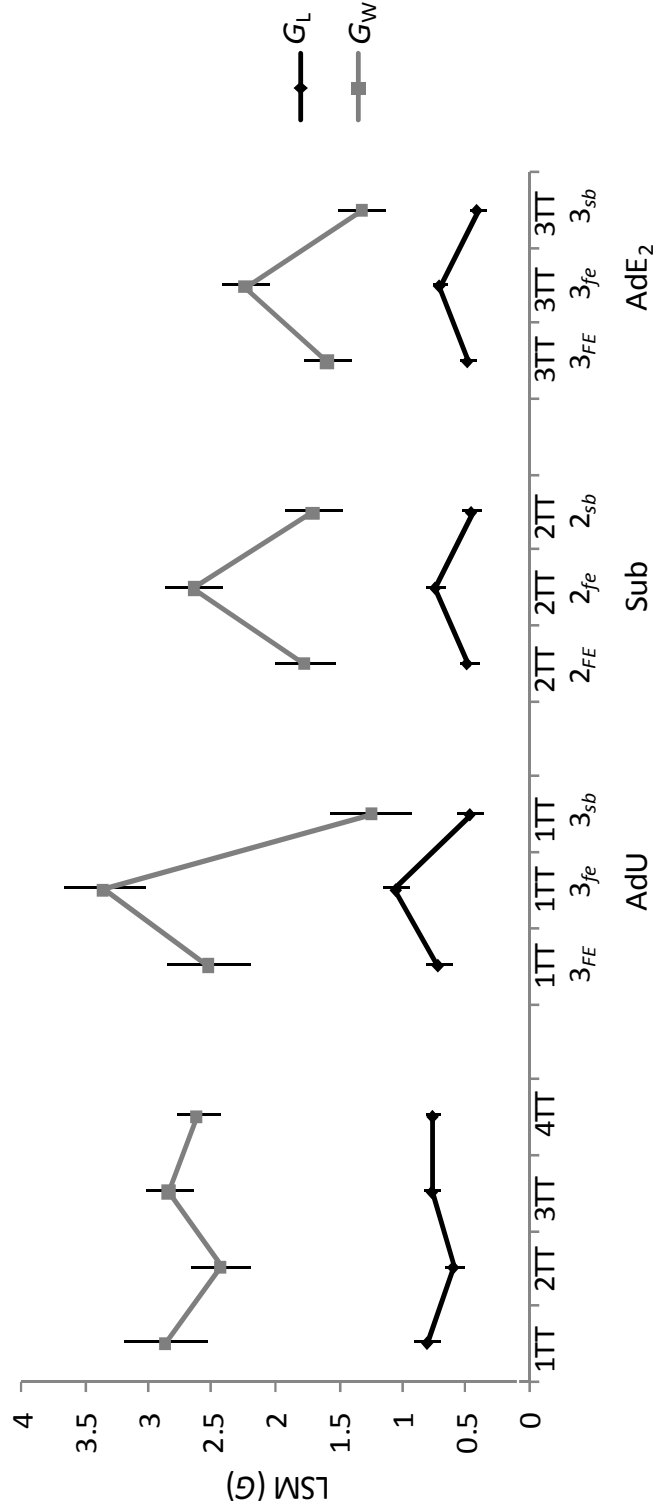
**Figure 1.** Depiction of fish species, types and sizes from syntopic populations (i.e. field) in the Eastern River system and those used in laboratory experiments. Two lines of *O. niloticus* were used in this study, feral (represented by ON<sub>FE</sub> and ON<sub>fe</sub>) and selectively bred (ON<sub>sb</sub>). The ON<sub>fe</sub> and ON<sub>sb</sub> used in experiments were sized according to the average total length ( $L_T^-$ , cm) of *O. niloticus* captured at field sites. The ON<sub>FE</sub> were size matched to the *T. trichopterus* (hereafter TT) captured at field sites. The three types on *O. niloticus* used in experiments are referred to as ON<sub>x</sub>. Fish were intraspecifically size matched ( $L_T \pm 0.01$  cm) within each treatment tank. The ON<sub>FE</sub> were interspecifically size matched to TT within each treatment tank. The TT, ON<sub>fe</sub>, and ON<sub>sb</sub> used in experiments represented the differential sizes of *T. trichopterus* and *O. niloticus* sampled in the field. The  $L_T^-$  and size range (cm) are given for animals captured in the field and on day 1 of the experiment.



**Figure 2.** Experimental tank schematic showing the shelter (labeled 1), automatic feeder (2), and water outlet (3), and area quadrants used to record fish position and behavior (a – g).

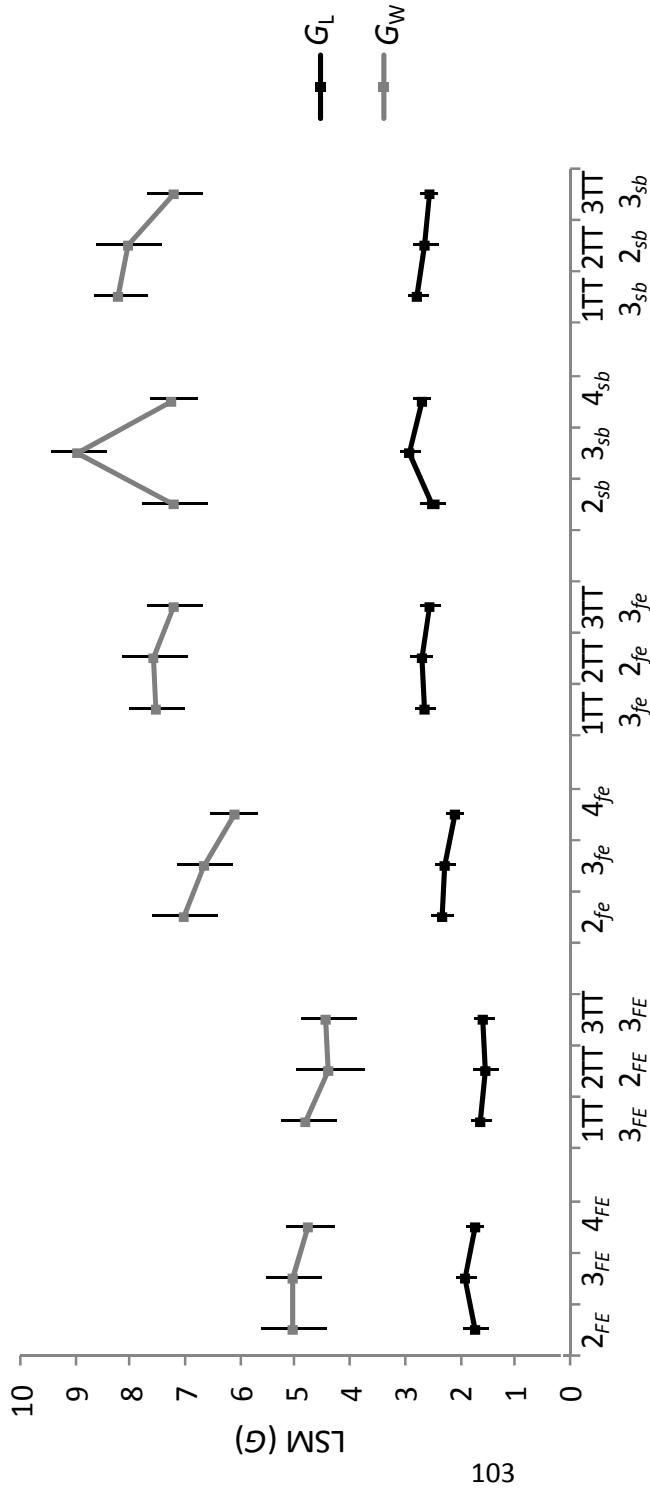
Density scheme	Single species treatments		Mixed species treatments
	<i>T. Trichopterus</i>	<i>O. Niloticus</i>	<i>T. Trichopterus + O. niloticus</i>
Additive Equal <sub>1</sub> (AdE <sub>1</sub> )	3TT	3ON <sub>x</sub> <sup>α</sup>	3TT + 3ON <sub>x</sub>
Additive Equal <sub>2</sub> (AdE <sub>2</sub> )	2TT	2ON <sub>x</sub>	2TT + 2ON <sub>x</sub> <sup>α</sup>
Additive Unequal (AdU)	1TT	3ON <sub>x</sub> <sup>α</sup>	1TT + 3ON <sub>x</sub>
Substitutive (Sub)	4TT	4ON <sub>x</sub>	2TT + 2ON <sub>x</sub> <sup>α</sup>

**Figure 3.** Design of experiments examining intra- and interspecific competition between *T. trichopterus* and *O. niloticus* for *T. trichopterus* (TT) only, mixed species treatments, and *O. niloticus* only treatments. We use ON<sub>x</sub> to represent *O. niloticus*-type, which has three levels: ON<sub>FE</sub>, ON<sub>fe</sub>, and ON<sub>sb</sub>. Therefore, there were 13 treatment levels for TT, and 18 for ON<sub>x</sub>. All single species ON<sub>x</sub> treatments and mixed species treatments were conducted with each level of ON<sub>x</sub>. Four replicates of each treatment were conducted. Duplicated treatments appear twice in the diagram (rectangles with α in the upper right hand corner), but only four replicates, rather than eight, were conducted for each ON<sub>x</sub> level. These duplicated treatments are shown on the diagram to illustrate all possible comparisons in the experimental analysis.



**Figure 4a.** Least squared mean (LSM) plots of the instantaneous growth rate for *T. Trichopterus* as length ( $\bar{G}_L$ ) and weight ( $\bar{G}_W$ ) in single (S) and mixed (M) species treatments for each density scheme and for each *O. niloticus*-type. Error bars represent  $\pm 1$  standard error. Due to space limitations, only the subscripts for each *O. niloticus*-type are given in the figure. See Appendix 2 for mean values and treatments that were significantly different as indicated by the Tukey-Kramer multiple comparison tests for  $\bar{G}_L$  and  $\bar{G}_W$ . See Figures 2 and 3 for abbreviated names and treatment descriptions, respectively.

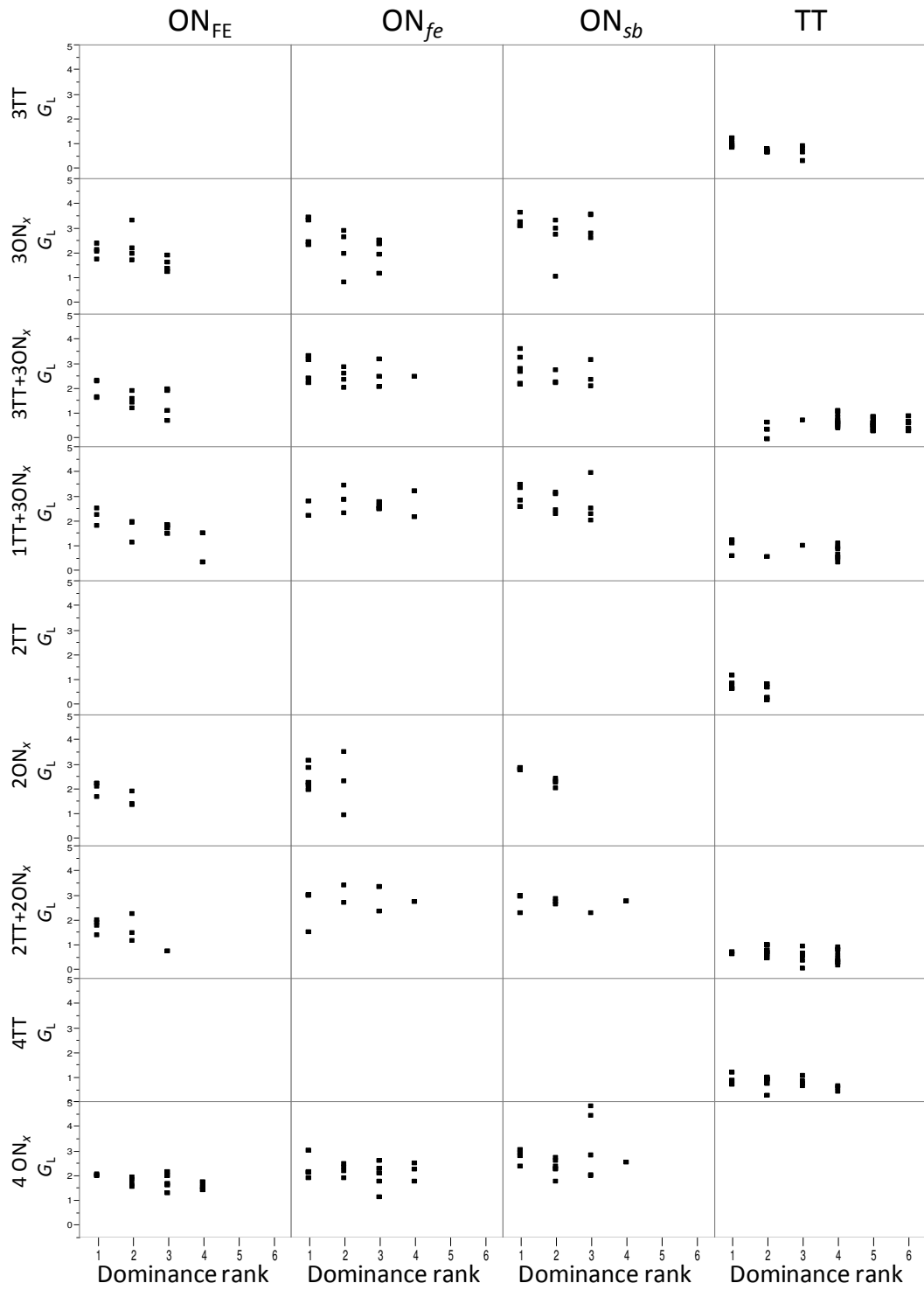




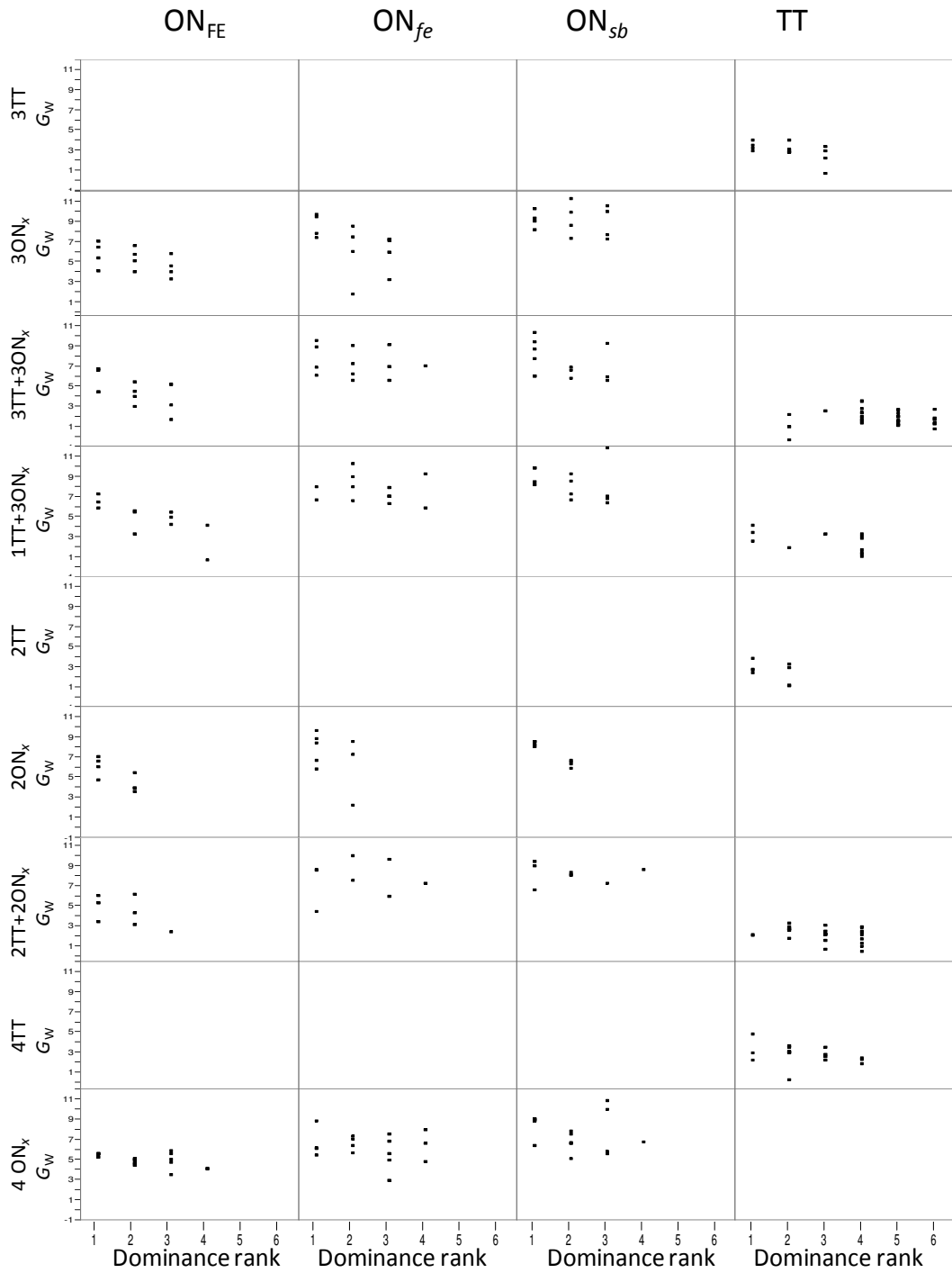
**Figure 4b.** Least squared mean (LSM) plots of the instantaneous growth rate for *O. niloticus* as length ( $\bar{G}_L$ ) and weight ( $\bar{G}_W$ ) in single (S) and mixed (M) species treatments for each density scheme and for each *O. niloticus*-type. Error bars represent  $\pm 1$  standard error. Due to space limitations, only the subscripts for each *O. niloticus*-type are given in the figure. See Appendix 2 for mean values and treatments that were significantly different as indicated by the Tukey-Kramer multiple comparison tests for  $\bar{G}_L$  and  $\bar{G}_W$ . See Figures 2 and 3 for abbreviated names and treatment descriptions, respectively.

**Appendix 1.** Overlay plots of the instantaneous growth rate of *T. trichopterus* and each *O. niloticus*-type for (a) length ( $G_L$ ) and (b) weight ( $G_W$ ) for individual fish in each dominance rank for each treatment. The dominant fish in each treatment tank was awarded rank 1, the subdominant rank 2, and subordinate fish are ranked 3 through 6 based on their total behavioral score (Table 1).

Appendix 1a



# Appendix 1b



**Appendix 2.** The mean instantaneous growth response for length ( $G_L$ ) and weight ( $G_W$ ) (Ricker 1979) and one standard error (SE) for each treatment in the ANOVA models. Levels not connected by same letter are significantly different as indicated by the Tukey-Kramer test for multiple comparisons.

## Appendix 2

### *T. trichopterus*

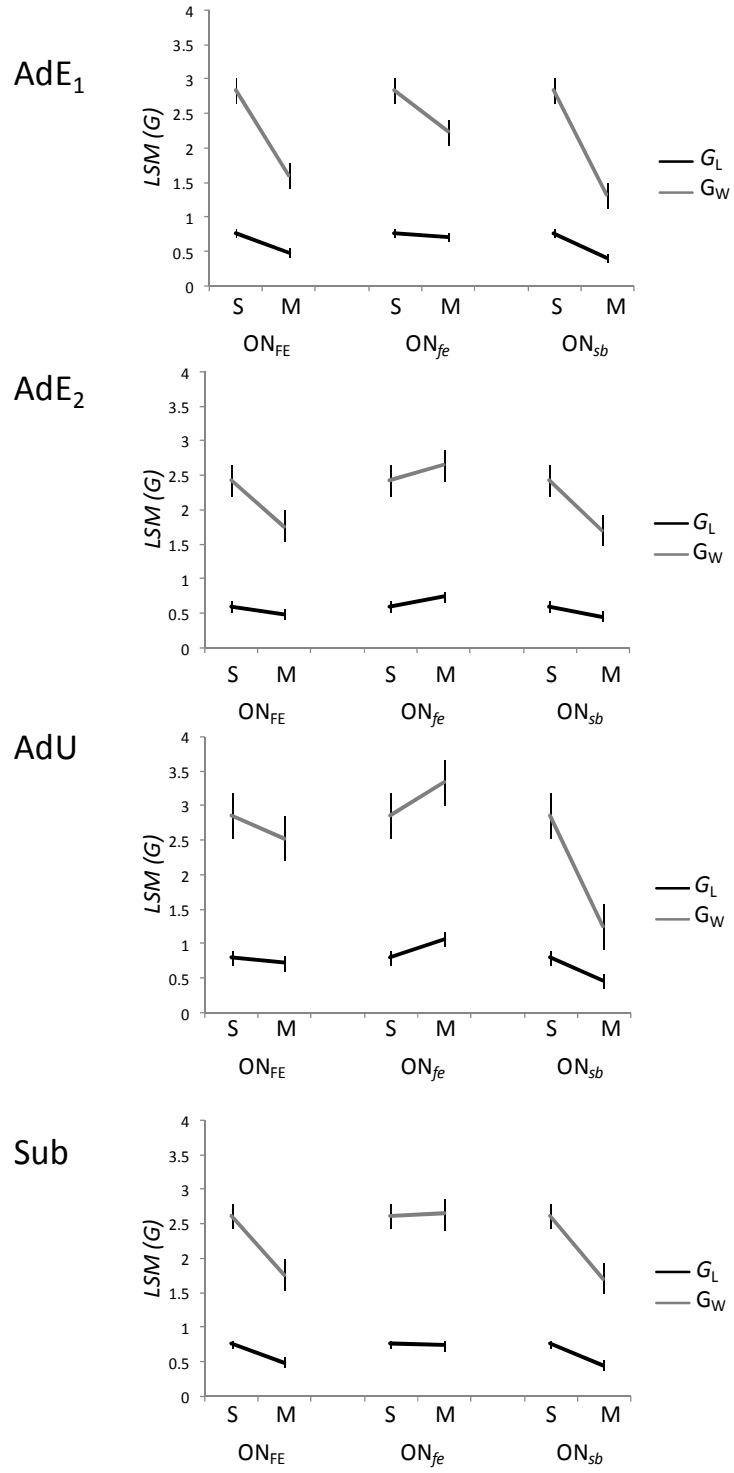
Density scheme	Treatment	$G_L$			$G_W$		
		$\bar{x}$	SE		$\bar{x}$	SE	
AdU	1TT	0.81	0.10	a b c	2.87	0.33	a b c d
AdE <sub>2</sub>	2TT	0.60	0.08	a b	2.43	0.23	a b c d
AdE <sub>1</sub>	3TT	0.77	0.06	b c	2.84	0.19	a b
Sub	4TT	0.76	0.06	a b	2.62	0.17	a b c
AdU	1TT + 3ON <sub>FE</sub>	0.72	0.10	a b c	2.54	0.33	a b c d e
AdU	1TT + 3ON <sub>fe</sub>	1.06	0.10	a	3.36	0.33	a
AdU	1TT + 3ON <sub>sb</sub>	0.46	0.10	b c	1.25	0.33	d e
AdE <sub>2</sub> /Sub	2TT + 2ON <sub>FE</sub>	0.49	0.08	b c	1.77	0.23	b c d e
AdE <sub>2</sub> /Sub	2TT + 2ON <sub>fe</sub>	0.74	0.08	a b c	2.65	0.23	a b c d
AdE <sub>2</sub> /Sub	2TT + 2ON <sub>sb</sub>	0.45	0.08	c	1.71	0.23	c d e
AdE <sub>1</sub>	3TT + 3ON <sub>FE</sub>	0.48	0.06	b c	1.60	0.19	d e
AdE <sub>1</sub>	3TT + 3ON <sub>fe</sub>	0.71	0.06	a b c	2.24	0.19	a b c d e
AdE <sub>1</sub>	3TT + 3ON <sub>sb</sub>	0.41	0.06	c	1.33	0.19	e

### *O. niloticus*

Density scheme	Treatment	$G_L$			$G_W$		
		$\bar{x}$	SE		$\bar{x}$	SE	
AdE <sub>2</sub>	2ON <sub>FE</sub>	1.74	0.23	b c d	5.04	0.60	c d e f g
AdE <sub>1</sub> /AdU	3ON <sub>FE</sub>	1.93	0.18	b c d	5.05	0.50	d e f g
Sub	4ON <sub>FE</sub>	1.75	0.16	c d	4.76	0.44	g
AdU	1TT + 3ON <sub>FE</sub>	1.65	0.18	c d	4.78	0.50	e f g
AdE <sub>2</sub> /Sub	2TT + 2ON <sub>FE</sub>	1.56	0.23	c d	4.38	0.60	f g
AdE <sub>1</sub>	3TT + 3ON <sub>FE</sub>	1.60	0.18	d	4.41	0.50	g
AdE <sub>2</sub>	2ON <sub>fe</sub>	2.35	0.23	a b c d	7.05	0.60	a b c d e f g
AdE <sub>1</sub> /AdU	3ON <sub>fe</sub>	2.28	0.18	a b c d	6.68	0.50	a b c d e f g
Sub	4ON <sub>fe</sub>	2.13	0.16	a b c d	6.12	0.44	b c d e f g
AdU	1TT + 3ON <sub>fe</sub>	2.65	0.18	a b	7.53	0.50	a b c d
AdE <sub>2</sub> /Sub	2TT + 2ON <sub>fe</sub>	2.72	0.23	a b c	7.61	0.60	a b c d e
AdE <sub>1</sub>	3TT + 3ON <sub>fe</sub>	2.56	0.18	a b c	7.23	0.50	a b c d e f
AdE <sub>2</sub>	2ON <sub>sb</sub>	2.50	0.23	a b c d	7.21	0.60	a b c d e f g
AdE <sub>1</sub> /AdU	3ON <sub>sb</sub>	2.93	0.18	a	8.98	0.50	a
Sub	4ON <sub>sb</sub>	2.70	0.16	a b	7.25	0.44	a b c d
AdU	1TT + 3ON <sub>sb</sub>	2.79	0.18	a b	8.22	0.50	a b
AdE <sub>2</sub> /Sub	2TT + 2ON <sub>sb</sub>	2.65	0.23	a b c d	8.04	0.60	a b c
AdE <sub>1</sub>	3TT + 3ON <sub>sb</sub>	2.58	0.18	a b c	7.22	0.50	a b c d e f

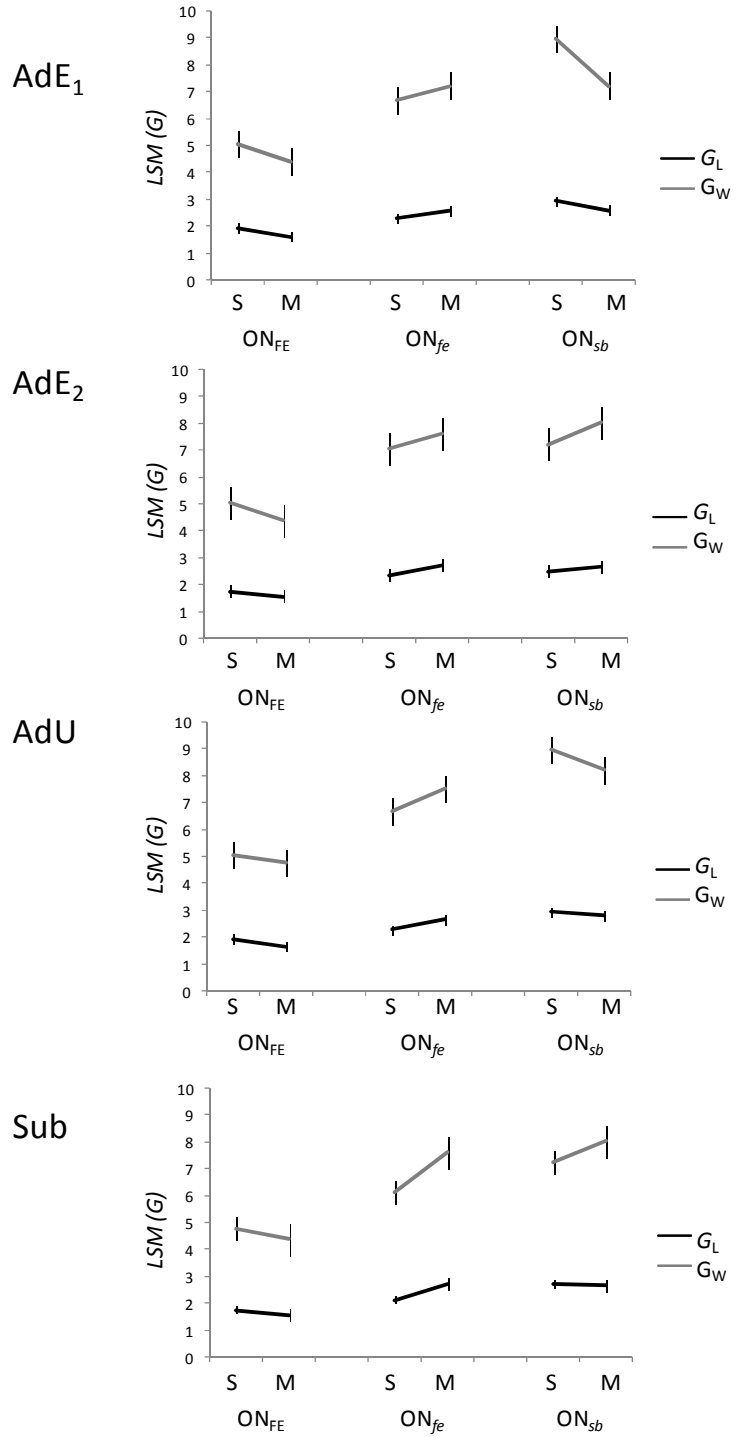
**Appendix 3.** Least squared mean (LSM) plots of the instantaneous growth rate for (a) *T. trichopterus* and (b) *O. niloticus* as length ( $\bar{G}_L$ ) and weight ( $\bar{G}_W$ ) in single (S) and mixed (M) species treatments for each density scheme and for each *O. niloticus*-type. See Figure 3 for descriptions of density schemes and treatments. Error bars represent  $\pm 1$  standard error.

Appendix 3a





Appendix 3b



## Epilogue

My dissertation research presented a step-by-step approach for assessing the potential for adverse ecological effects of a non-native fish using a fault tree of events. Events in the fault tree follow logic expressions necessary for a non-native fish species to cause an adverse change in the native fish assemblage (Figure 1, Prologue). My field research (Chapter 1) documented exposure events at levels I, II, and III (Figure 1, Prologue). Field sites were shallow streams with water quality, depth, and clarity that was conducive to backpack electroshocking. In these sites I found that non-native *Oreochromis niloticus* live in the natural environment with native fishes, including *Channa* spp. The *O. niloticus* that I captured were very small, and were therefore likely hatched in the wild from feral parents. In addition, *O. niloticus* relied on shared resources with native species. They had high dietary and habitat overlap with native species, and they did not prey on fish. These exposure event findings show that higher level effects events on the fault-tree are likely to occur.

I used my findings from the field, e.g., choice of species and strains, and relative fish sizes and densities, to inform my laboratory experiments. In the laboratory (Chapter 2), I examined both the mechanism and outcome of intra- and interspecific competition at varying fish densities. I found that dominance hierarchies were readily established, and *O. niloticus* was the most dominant individual in 86.1% of all mixed species treatment tanks. As a species, *O. niloticus* held higher dominance status over all *T.*

*trichopterus* in 67.7% of all mixed species treatment tanks. Dominance status was important because it had consequences for fish growth. Dominant fish had primary access to the food source, and grew more than fish with lower dominance status. Specifically, subordinate *T. trichopterus* grew significantly less than subdominant and dominant *T. trichopterus*, and subdominant and subordinate *O. niloticus* had reduced growth compared to dominant *O. niloticus* individuals.

In the laboratory, I found that not all types of *O. niloticus* were the same when it came to their effect on the growth of *T. trichopterus*. The native fish, *T. trichopterus*, grew slightly more in mixed species treatments containing feral *O. niloticus* that were in the size range found in the field ( $ON_{fe}$ ) than when alone. In contrast, *T. trichopterus* grew significantly less when in mixed species treatments containing *O. niloticus* selectively bred for enhanced growth ( $ON_{sb}$ ) than when alone. This finding has important implications for the use and distribution of selectively bred *O. niloticus* relative to feral *O. niloticus* in Thailand, throughout their naturalized range, and within the native range of *O. niloticus*.

I found that, through the mechanism of interference competition, this particular line of  $ON_{sb}$  resulted in significantly decreased growth of the native species. This risk assessment finding is site- and line-specific, and therefore not generalizable to all situations. However, this finding clearly raises the need to assess potential adverse effects on native species by interference competition in proposed introductions of

growth-enhanced lines of *O. niloticus*. This issue should be explicitly addressed within an environmental risk assessment, using data from similar empirical experiments or uncertainty analyses that address the relative effect(s) of competition of selectively bred lines and feral lines on selected native species under site-relevant conditions. In the native range of *O. niloticus*, it would be especially salient to test the effects of selectively bred lines on wild *O. niloticus* and other native fishes prior to any introductions. If empirical data cannot be collected, the risk assessment should apply uncertainty analysis, such as sensitivity analysis or qualitative modeling, to explicitly treat interference competition as a source of model uncertainty throughout the risk assessment (Hayes et al. 2007).

My methodologies and findings lay a solid foundation for ongoing and iterative risk assessment research and highlight the importance of site specific data for ecological risk assessment. I did not intend for the results presented here to be the final chapter in the risk assessment of *O. niloticus* in Thailand. The laboratory studies were not designed to definitively answer how *O. niloticus* affect *T. trichopterus* in the wild, and the field studies represent a selected set of habitats that were amenable to my sampling gear. To gain a deeper understanding of how competition affects these species in the wild, a similar experimental design could be applied in semi-naturalized field settings in controlled ponds or exclusion experiments in streams. Findings from these experiments could then be used to inform a further series of field investigations to gain a broader understanding of *O. niloticus* effects across more diverse environments and life stages.

Based on findings from these field studies, further laboratory studies could be done to elucidate the effects of competition. Such iterative studies will help to quantitatively estimate the probability and magnitude of establishment of *O. niloticus* and resource overlap with native species in a site-specific ERA of *O. niloticus*, and these studies will assist in the risk assessment of any introduced fish.

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