LIFE HISTORY AND ENVIRONMENTAL TOLERANCE

OF THE INVASIVE ORIENTAL WEATHERFISH (*Misgurnus anguillicaudatus*)

IN SOUTHWESTERN IDAHO, USA

by

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DEDICATION

This thesis work is dedicated to my friends and family who have aided and
supported me throughout this pursuit. Special thanks are addressed to my parents Donald
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ABSTRACT

Invasive fish species have been identified as a major threat to aquatic biodiversity world-wide. The most successful of these invaders share several life history characteristics such as long lifespan, high fecundity, multiple reproductive events, generalized omnivorous diet, and tolerance for a wide range of environmental conditions. Although many studies have focused on well-known and economically costly invaders, there are many invasive fish about which very little is known. In this series of studies, I describe some life history characteristics of one such invasive fish, the oriental weatherfish (*Misgurnus anguillicaudatus*). I collected 586 specimens from water bodies connected with the lower Boise River, Idaho, USA. For the first study, I dissected 237 of these individuals and used morphometrics, ova counts, and otolith ageing to build a life history profile of this invasive population. I found a 1:1 sex ratio of sexually mature males and females. Young of the year displayed rapid growth to sexual maturity within one year of life and fish lived to six years of age in the wild. Upon reaching maturity, the fish became capable of reproduction during two predicted spawning events over a protracted spawning season (June through October). Female fish had the capacity for releasing up to 40,000 eggs per spawning season. For the second study, I examined the stomach contents of the dissected fish and used gravimetric, frequency, and abundance data to determine the fish's diet. Oriental weatherfish were eating a generalized diet of aquatic invertebrates and detritus. For the third study, I used a temporally extended Critical Thermal Minimum (CTmin) approach to find the lowest water temperature that

the fish could survive. Fish survived exposure to subfreezing water temperatures and direct contact with ice. I used logistic regression to estimate the CTmin of this sample of oriental weatherfish as -1.76°C. This series of studies shows that the oriental weatherfish possesses many hallmark characteristics of other successful invasive fishes. These characteristics, coupled with ongoing dispersal through the aquarium pet trade make the oriental weatherfish an ideal invasive species. Currently, the oriental weatherfish is invasive in at least 10 countries throughout the world, and further research into the impacts that the fish has on native faunal communities is needed.

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CHAPTER ONE: INTRODUCTION

Biodiversity is being threatened world-wide, by the introduction and spread of

invasive species (Lockwood et al. 2000; Olden et al. 2004; Keith et al. 2009; Villeger et

al. 2011). Most organisms that are intentionally or accidentally introduced outside of their

native range fail to establish self-sustaining populations (Mack et al. 2000). Others,

however, take hold and proliferate within their new surroundings with varied and often

unintentional results (Moyle and Light 1996, Simberloff and Stiling 1996). These species

often become invasive and cause disruption of native communities through competition

for common resources, habitat modification, predation, and introduction of parasites and

pathogens (Mills et al. 1994, Mack and D'Antonio 1998, Telfer and Bown 2012).

Consequently, these disruptions incur billions of dollars in annual losses and damage

around the globe (Pimentel 2005). This is no less true in freshwater aquatic ecosystems.

Aquatic invasive species have long been characterized by ecological horror stories

of introduced organisms wreaking havoc upon native communities through direct

exploitation, competition, habitat degradation and pathogen introduction. For example,

when introduced into Lake Victoria, East Africa, as a potential commercial fishery, the

Nile perch (*Lates niloticus*) decimated already overexploited native fish populations

through predation (Ogutu-Ohwayo 1990). Accordingly, aquatic invasive species are

widely held as ecologically and economically detrimental (Moyle and Light 1996,

Pimentel 2005). However, the effects of these invaders have been primarily characterized

based on case studies of iconic species such as Nile perch, zebra mussels (*Dreissena polymorpha*), Asian carp (*Cyprynus idella*, *Cyprinus carpio*, *Hypophthalmichthys moltrix*) and other highly publicized species (Vitule et al. 2009).

Throughout the world, many invasive species have established populations that have had little or no documented negative ecological impact (Gozlan 2008). These species may not only yield no negative impact in their introduced ranges; they may serve a beneficial capacity, becoming a food source for native species. For example, the non-native round goby (*Neogobius melanostromus*) introduced into the Laurentian Great Lakes is a known predator of the economically costly zebra mussel (Ghedotti et al. 1995, Ray and Corkum 1997). In turn the goby has become an important food source for native and sport fish populations (Steinhart et al. 2004), thus transferring biomass from an invasive bivalve species through the trophic food web to native and desirable species. The goby has even become a primary diet component of the endangered Lake Erie water snake (*Nerodia sipedon insularum*; King et al. 2006). While positive impacts of introduced species are admittedly rare and controversial (Gozlan 2008, Vitule et al. 2009, Davis et al. 2011, Simberloff 2011), this example highlights the need to understand the ecology of lesser known introduced and invasive species before their impacts can be determined. The oriental weatherfish (*Misgurnus anguillicaudatus*) is one such invasive species that is in need of study. Until the ecology of this fish is understood, its impacts within invaded ranges will remain unknown.

The oriental weatherfish is native to eastern Asia, ranging from India to Vietnam, and north to southern Russia. Within its native range the fish is commonly raised as a food product, used as live bait for sport fishing, and sold internationally as an exotic pet

fish. The aquarium trade may have aided the range expansion of the fish (Chang et al. 2009; Strecker 2011), and oriental weatherfish populations are currently reported in at least ten countries. These include the Philippines, Turkmenistan, Spain and Italy (Simon et al. 2006; Franch et al. 2008), Germany (Freyhof and Korte 2005), Australia (Lintermans et al. 1990; Arthington and Bludhorn 1995), Mexico (Contreras and Escalante 1984), and the United States (Fuller et al. 1999; Tabor et al. 2001; Ashton and Ciccotto 2010). Range expansion of the fish appears to be continuing through continued introductions and most recently, populations have been documented in the Netherlands (van Kessel et al. 2013) and Brazil (Abilhoa et al. 2013).

Within the United States, populations of oriental weatherfish have been reported in 13 states, ranging from subtropical locales such as Florida and Hawaii, to more northern climes; e.g., Washington and Michigan (Fuller et al. 1999; Tabor et al. 2001; Simon et al. 2006; Ashton and Ciccotto 2010). Even with this broad known range, very little is known of the fish's impacts on native biota or its ability to survive in such a broad range of habitats.

Anecdotal accounts of oriental weatherfish found burrowed into in the muddy substrate of drying water bodies has afforded them a reputation of a survivalist fish, that is able to disperse over dry land (Burchmore et al. 1990, Lintermans 1990, Corfield et al. 2007). Empirical studies show that the fish can survive drought conditions by aestivation in the soil (Koetsier and Urquhart 2012). The fish is also a facultative air breather that can survive hypoxic conditions through gulping air at the water surface and passing it through its highly vascularized hindgut (McMahon and Burggren 1987). During atmospheric respiration, the fish volatilizes nitrogenous wastes as gaseous ammonia

through its skin (Chew et al. 2000, Ip et al. 2004). While these fantastical descriptions conjure an exciting image of the invasive potential of this cryptic fish, many of the most basic life history characteristics of the oriental weatherfish remain undescribed within its invasive range. Thus, it is unclear as to how these traits are actually utilized within invaded environments to facilitate the fish's survival and establishment of new populations.

Given its establishment in so many disparate locations around the globe, surprisingly little information exists about the ecology or impacts of this fish on native ecosystems. However, a few studies indicate the possible ecological impacts this fish may be having on native biota. In 1986, Australia banned the importation of oriental weatherfish due in part to concerns over declines in native fish populations in the areas where escaped oriental weatherfish were found (Burchmore et al. 1990, Lintermans 2002, Corfield et al. 2007). Concern may also be warranted in Germany, where an endangered native congener, the European weatherfish (*M. Fossilis*) may be susceptible to further habitat loss due to its invasive sister species (Meyer and Hinrichs 2000). Additionally, the oriental weatherfish is known to be the vector of introduction of at least one monogaean parasite (Dove and Ernst 1998), and carries the LV1 Birnavirus, a fish virus related to the highly destructive Infectious Pancreatic Necrosis Virus (Wolf 1988; Lintermans et al. 1990). While these potential threats to native fauna may be substantial, other effects may exist of which we are currently unaware.

In Idaho, USA, the oriental weatherfish was first documented in 1951, in an irrigation canal near the town of Eagle (Courtenay et al. 1987). Since that time, the fish has resided within river side channels and the man-made agricultural canal systems and

irrigation ponds for more than 50 years with no documented impacts on native biota. However, within the Boise and the Snake River systems, the oriental weatherfish may utilize and compete for important resources such as aquatic invertebrate prey, or habitat that is also utilized by native fish species (Keller and Lake 2007). Alternatively, these introduced fish may serve as a food source for native cyprinids and economically important introduced centrarchid species (*Micrpterus salmoides, Micropterus dolimieu*; IDFG personnel, personal communication). Thus, as seen with the round goby, an invasive species may provide some benefit within its invaded ecosystem.

Before determination can be made regarding the possible impacts (whether positive or negative) that oriental weatherfish may have on native biota, it is imperative to know the basic life history of the species. For example, to estimate the ability of the oriental weatherfish to increase its population size and expand its invaded range over time, we must first know details of the population dynamics such as the sex ratio of the current population, when the fish reaches maturity, and when and how much it reproduces within these invaded regions. In order to estimate the potential limits of the invasive range of the oriental weatherfish or predict where it may become invasive, we must know what habitat conditions may limit the fish's survival. In order to determine if competition for prey is taking place with native fauna, we must know what the oriental weatherfish eats withi4n its invaded range. Each of these life history characteristics helps to build a composite image of this cryptic invader. From this image we can begin to discern what actual effects the oriental weatherfish may have on native faunal communities.

With these needs in mind, I embarked on a series of studies designed to define several important life history characteristics of oriental weatherfish from an invasive population within the Boise River system, in southwestern Idaho, USA. In the first study, I explore the sex ratio, patterns of age and growth and the reproductive capability of the population. I also use fecundity, body morphometrics and reproductive seasonality to predict oriental weatherfish spawning timing and periodicity. In the second study, I examine the ability of the oriental weatherfish to tolerate subfreezing water temperatures and explore the effect that seasonal water temperatures and habitat conditions may have on the fish's ability to overwinter. In the third study, I identify the diet of Boise River oriental weatherfish as determined from stomach contents.

From the life history traits and minimum temperature tolerance limits revealed in this series of studies, I form a composite image of a highly successful invasive species. The oriental weatherfish emerges as a fish that displays characteristics common to other successful invasives including long life, early maturity, high fecundity, and multiple spawning events (Marchetti et al. 2004). Added to this is the fish's ability to survive seemingly inhospitable habitat conditions, including subfreezing temperatures and ice exposure. Finally I characterize the oriental weatherfish as a generalist omnivore that may retain a carnivorous diet later in life than seen within its native range. Consequently, the impacts of this cryptic invader upon native faunal communities may be significant and I recommend that the oriental weatherfish become a target invasive species of further ecological research.

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CHAPTER TWO: AGE, GROWTH, FECUNDITY AND SPAWN TIMING WITHIN

AN INVASIVE POPUALTION OF ORIENTAL WEATHERFISH (*Misgurnus*

anguillicaudatus) IN AND AROUND THE BOISE RIVER, IDAHO, USA

Abstract

The oriental weatherfish (*Misgurnus anguillicaudatus*) has become established in many areas outside of its native range. Within invaded regions, very little is known of the life history or ecological effects of this fish and it remains a secretive and unstudied resident of many parts of the world. Here, we report on some of the basic life history characteristics of an invasive population of oriental weatherfish collected from the Boise River, southwestern Idaho, USA. We collected 586 fish between May- 2008 and July-2009 using minnow traps baited with commercial dog food, from six sites within irrigation canals and agricultural or ornamental ponds connected with the lower Boise River. From these collections, 237 individuals were euthanized, dissected and analyzed to determine the population sex ratio, patterns of age and growth, sexual maturity, fecundity, breeding season and spawning frequency. Within our study area, oriental weatherfish lived 5 - 7 years, reaching sexual maturity within the first year of life. Females remained fecund throughout their lifetime, and spawned twice per year, with young produced in the late spring (May) and early autumn (October) seasons. Our investigation is the first to document the life history of a successfully established population of oriental weatherfish outside of its native range. As such, the results of this

study provide a baseline by which the scientific community can begin to assess the potential impacts of this poorly understood species on native biota.

Introduction

Worldwide, biodiversity is being threatened by many anthropogenic factors, including introduced and invasive species (Lockwood et al. 2000; Olden et al. 2004; Keith et al. 2009; Villeger et al. 2011). Aided by increased global interconnectivity, including international commerce and intercontinental transport of ornamental pet organisms, the spread of non-native biota is increasing. The associated ecological and economic impacts in both terrestrial and aquatic ecosystems are likewise escalating (Pimentel et al. 2005; Olden et al. 2006; Hulme 2009; Strecker et al. 2011). In the United States alone, an estimated 138 non-native fish species have been introduced. Many of these have become established and are now considered a permanent part of the ecosystem (Taylor et al. 1984; Moyle and Light 1996; Mooney and Cleland 2001; Pimentel et al. 2005). Within aquatic communities, studies of invasive species have focused primarily on top predators including those introduced for sport fisheries (e.g., salmonids, centrarchids) or species with highly visual and economic impacts (e.g., water hyacinth, *Eichhornia sp*; Eurasian water milfoil, *Myriophyllum spicatum*; zebra mussels, *Dreissena polymorpha*). However, there are many successfully established aquatic organisms, about which very little is known. These lesser known species may lack the dramatic aesthetic or economic impact of more publicized invaders; however, their ecological impacts may be nonetheless significant where introduced.

The oriental weatherfish (*Misgurnus anguillicaudatus*) is one such cryptic invader. This fish is native to eastern Asia, with range spanning from Myanmar and Viet Nam through southern Siberia, including the Japanese archipelago. Established populations are now reported in at least eight countries outside of its native range, including the Philippines, Turkmenistan, Italy, and Spain (Freyhof and Korte 2005; Simon et al. 2006; Franch et al. 2008). In Germany, an endangered native congener (*M. fossilis*) appears to be losing habitat to its introduced sister species (Meyer and Hinrichs 2000; Freyhof and Korte 2005), and laboratory studies and field observations in Australia indicate decreases of native populations where oriental weatherfish have successfully established (Lintermans et al. 1990; Arthington and Bludhorn 1995). These losses may be due to competition for invertebrate prey (Keller and Lake 2007), or direct predation of native fish eggs and larvae (Logan et al. 1996). As a result, importation of the fish has been banned by law since 1986 (Burchmore et al. 1990; Lintermans 2002; Keller and Lake 2007). In North America, oriental weatherfish have become established in Mexico (Contreras and Escalante 1984) and 13 of the United States, ranging from subtropical locales such as Florida and Hawaii, to more northern climes; e.g., Washington and Michigan (Fuller et al. 1999; Tabor et al. 2001; Simon et al. 2006; Ashton and Ciccotto 2010). In Idaho, the first reported oriental weatherfish was collected near the town of Eagle, in 1951 (Courtenay et al. 1987). Since that time, a self-sustaining population has become established and is thriving in the man-made habitats of irrigation ponds and canals associated with the lower Boise River (Urquhart and Koetsier 2011). Furthermore, the oriental weatherfish is known to harbor 2 monogean parasites (Dove and Ernst 1998) and at least one fish virus of concern to economically important and endangered

salmonids (LV1 Birnavirus, a close relative of Infectious Pancreatic Necrosis Virus; Wolf 1988; Lintermans et al. 1990).

The aquarium pet and ornamental fish trade provides a source of oriental weatherfish around the globe (Chang et al. 2009; Strecker 2011), yet we know virtually nothing of the potential ecological impacts of continued introduction, establishment and spread of the fish. Given the difficulty of eradicating any species once established (Vander Zanden et al. 2009; Horns 2010), the oriental weatherfish should be viewed as a permanent occupant within the freshwater faunal communities where its introduction has occurred. As such it is important to understand the basic ecology of this new resident in order to discern the potential impacts its presence may have, and to determine if control or eradication is warranted or even possible (Simberloff 2003).

To date, accounts of oriental weatherfish in the United States consist of simple species encounter records with limited habitat description and meager discussion, if any, of the ecology or potential interactions of this fish with native biota. In fact, before the potential impacts of oriental weatherfish can be studied, an understanding of the life history of the fish within its non-native range is needed. Consequently, our research focused on six fundamental life history components (sex ratio, patterns of age and growth, sexual maturity, fecundity, breeding season and spawning frequency) of oriental weatherfish taken from ponds and irrigation canals within the lower Boise River drainage, near Boise, Idaho, USA. From these life history parameters we composed a demographic profile of oriental weatherfish that is consistent with previous characterizations of other successful invasive species. Given its current level of

establishment around the globe, we also suggest that the oriental weatherfish be further investigated as a world-wide invasive species of concern.

Methods

<u>Sample Collection</u>

Between May- 2008 and July- 2009, we collected 586 oriental weatherfish from six water bodies (ponds and irrigation canals) with a seasonal connection to the Boise River (for collection locations, GPS coordinates, and voucher specimen records see Urquhart and Koetsier 2011). We placed vinyl coated, steel mesh minnow traps at plausible collection sites based on published habitat descriptions (Allen 1984; Tabor et al. 2001; Freyhof and Korte 2005) and Idaho Department of Fish and Game records (IDFG personnel, personal communication). Traps measured 42 cm x 22 cm with a 2.5 cm opening at the apex of an inverted cone in each end. Each was outfitted with a thermograph data logger connected to the outside, and a small surface buoy for relocation. Thermographs recorded water temperature once each hour or once every 2 hours (depending on location and data logger storage capacity) for the duration of collection at each site and were periodically replaced so that data could be downloaded in the laboratory. We baited each trap with approximately 30 g of dry commercial dog food, and one or two traps were placed at each collection site.

Traps were submerged upon the substrate and water depth of each trap varied based on water body such that depth at the internal opening ranged from approximately 10 cm to 1.5 meters below the water surface. Traps were removed from sampling sites

and relocated to new collection areas during the sampling period as dictated by lack of successful trapping, seasonal water levels or site accessibility. One trap was lost to unknown causes at a single site and daily temperature data for that site was also lost. No fish had been collected from that site and we did not replace the lost trap. Two successful collection locations remained inundated throughout our sampling period (seasonal irrigation canal, IDFG hatchery outlet settling pond) and traps remained at those locations throughout the entire year.

We checked traps every 4 - 10 days throughout the entire sampling period, and all collected fish were removed to the Boise State University, Aquatic Ecology laboratory. A screen mesh was sewn into a single trap to target juvenile fish in a single trapping location. All other locations were sampled using traps as described above.

In the laboratory, the sex of each fish was determined utilizing pectoral fin morphology following the methodology of Urquhart and Koetsier (2011). All fish were wet weighed to the nearest 0.1 g and measured for total length (TL in mm). Of the 586 collected fish, 237 were euthanized using a dilution of tricaine methanosulfonate (MS222) in freshwater and dissected immediately or frozen at -4°C and later, thawed for dissection. Eight fish were found desiccated in a trap that had been removed from the water body. These fish were sexed, but not weighed or measured and only included in the population sex ratio analysis. The remaining 349 fish were relocated to several live resident fish tanks (43 x 73 x 37.5 cm) in a temperature and humidity controlled greenhouse on the Boise State University campus; air temperature was held at 20°C, and all tanks had pour-over and under-gravel filters, and air-stone aeration. Resident fish were fed daily on commercial fish food by automatic timer feeders and tanks were cleaned

weekly of excess algae and maintained for constant water level. Resident fish were included in analyses for which sex or body size were factors, but were excluded from analyses involving age.

<u>Dissection</u>

Euthanized fish were incised from the vent forward through the pectoral girdle. The alimentary canal was removed intact by excision at the vent and the foregut just forward of the pectoral girdle. Heart, liver and extraneous connective tissues were removed and discarded. Intact digestive tracks were placed into glass vials in 70% ethanol. Gonads were removed intact by separation of the connecting membrane and removal of any other body tissue, then wet weighed to the nearest $1/10^{th}$ of a milligram. Ovaries were easily identifiable as red-brown to orange paired sacks and were often full of developing or vitellogenic ova. Testes were identified as white to clear, paired thin sacks and were often removed intact with the kidneys.

To obtain otoliths we split the head of each fish using small dissection scissors, cutting the skull in half from the mouth to above the gill arch. By laying the 2 halves open, while still connected to the body and the lower jaw, we could access the otoliths to either side of the cut. The lapillus otolith pair (Secor et al. 1991; Vilizzi and Walker 1999; Escot and Granado-Lorencio 2001; Morioka and Kaunda 2003) was removed and placed into scintillation vials containing 70% ethanol.

Post dissection, all fish carcasses were placed in zipper-sealed plastic bags, labeled and stored in a freezer at -4°C in the event that tissue sampling was desired later.

Otolith Ageing

To determine fish age, we first cleaned any remaining vestibular tissue from each otolith using 70% ethanol and dissecting pins under the aid of a dissecting microscope (Stemi 2000-C, Carl Zeiss). One of each otolith pair was mounted in epoxy using a half pour protocol obtained from Idaho Department of Fish and Game (L. Mamer, personal communication). The second of each pair was stored in 70% ethanol for use in the event the first otolith was lost, damaged or deemed unreadable. We mixed a batch of Buehler Epo-Thin® epoxy per the product directions and filled the small bullet-shaped wells in a set of Pelco® 105 Flat Embedding Molds to ½ capacity. After the epoxy cured, molds were filled to full capacity and otoliths were placed into the uncured epoxy, rostrum forward. Air bubbles clinging to the otoliths were removed using dissection pins. The molds were left to cure for 24 hours, after which the hardened epoxy blocks containing the otoliths were removed. We cut transverse sections from each mounted otolith using a Buehler IsoMet® low speed saw. The blade was centered at the crest of the otolith and moved rostraly 152.5 µm where the first cut was made. The sample was then moved such that the second cut resulted in a 305.0 µm transverse section encompassing the larval focus of the otolith suspended in epoxy. We used a dissecting microscope (EZ4D, Leica Microsystems, Ltd.) with an integral digital camera to examine, capture and store images of the otolith sections (Figure 2.1) for each fish. Each section was immersed in clarifying solvent and annular rings were counted to determine age in years. With no published studies of annular growth patterns for the oriental weatherfish available, we adopted the conventional practice of combining a single opaque growth ring set with a single translucent growth ring set in a banding pair to equate as one year of growth (Jearld 1983;

Devries and Frie 1996; Schill et al. 2010) as is typical of fish collected from seasonally

warm/cold environments. Fish otoliths varied as to banding pattern appearance, such that

the first band was equally likely to appear as opaque or translucent and variable in band

thickness. Where the larval focus was encircled by a translucent band, the following

complete opaque band was used as the end of the first annular pair. Where the first band

was opaque, the subsequent completed translucent band was then considered the outside

of the first annulus. The same convention was employed where the first band was

relatively thinner than subsequent bands, such that regardless the diameter of a band or

band pair, a single opaque and translucent band set was counted as one year of growth. At

the outside edge of the otolith section, if an associated pair of bands was not clearly

complete the incomplete set was added to the age as a "+" denoting that the fish was as

old as the total pairs of bands, plus some additional age time. Thus a fish with a total of 5

bands (3 opaque, 2 translucent) was considered a 2+ year aged fish for the purposes of

record keeping. However, for age analyses, all additional age indicated by the "+" was

disregarded and all fish were categorized as their full year ages regardless of the breadth

of additional incomplete band pairs.

<u>Age and Growth</u>

In attempting to create a predictive age/growth model that would allow non-lethal

age estimation of a population, TL and body weight were compared between ages using

one-way analysis of variance (ANOVA) with means comparisons. Sexes were separated

to further explore the growth relationships and ANOVA with means comparisons of TL

between sexes at each age provided a composite view of the growth patterns. These and

all subsequent statistical analyses were conducted using SAS v.9.2 (Cody and Smith

2006).

<u>Fecundity</u>

Gonadosomatic Index (GSI = (Gonad wet weight/Total weight) x 100) and Fulton

Condition Factor (FCF = (Body weight/TL^3) x 1000) were calculated for each fish using

standard techniques (Busacker et al. 1990; Anderson and Neuman 1996). Residual plots

of Female GSI versus collection date (Julian) showed evidence of a potentially cyclic,

time-based pattern during preliminary analyses; however, a time series analysis of this

relationship was not possible due to unequal time between collection dates. Instead, GSI

values were examined versus collection date in a standard linear regression to explore

spawn timing and synchronization within the population. Fecundity, spawn timing and

synchronization were further explored using samples of ovaries taken from dissected fish.

Approximately 1cm^3 samples were removed from each ovary-pair, such that both

ovaries were represented within each sample. These were fixed in a modified Davidson's

fixative (3:2:1:3 ratio of 90% ethanol, formalin, glacial acetic acid, deionized water) and

shaken vigorously to separate ova from the surrounding tissue. Where the proposed 1cm^3

sample exceeded actual gonad volume, the entire ovary-pair was used. We assigned each

fish as gravid, partially spent, spent, or immature based on the overall gonad appearance

and presence of primary, vitellogenic or atretic oocytes, or post ovulatory follicles.

Different staged oocytes preserved in Davidson's fixative appeared distinctly different

from each other. Primary oocytes appeared as small spheres with heavy staining around

the perimeter and a non-stained "hollow" center. Vitellogenic oocytes appeared as full,

"robust" spheres with a clear perimeter and solid yellow interior. Atretic oocytes

appeared as flaccid spheres, having lost turgidity, with the yellow center in the process of breaking down. Empty (post-ovulatory) follicles appeared as loose sacs of clear tissue that had lost their contents (Pollard 1972; Murua et al. 2003; Dadzie and Abou-Seedo 2008).

Each fixed sample was examined under a dissection scope at 45x to 60x power and vitellogenic oocytes were counted. Samples were dried in a wet/dry oven at 65°C for a minimum of 24 hours and dry weighed. We calculated the number of ova per gram of dry weight in each sample. The remaining (unsampled) ovary tissue was added to its corresponding weighed sample and dried for a minimum of 48 hours. The dry weight of each whole ovary-pair (counted sample + unsampled tissue) was determined and multiplied by the sample value ova per gram unique to each fish to extrapolate the number of vitellogenic oocytes per each ovary-pair (total ova per fish). These fecundity values were natural log transformed (x+1) and regressed against whole ovary-pair weight of 67 fish. The resulting polynomial regression equation that best fit these data was used to estimate number of mature ova per fish of 60 additional specimens whose ovaries were weighed but not sampled (Klibansky and Juanes 2008). Finally, resulting log transformed fecundity estimations were exponentiated [('value')^e -1] to determine the whole number ova estimates for each fish.

Ova estimates per fish were regressed versus body weight to determine reproduction potential of each female. Ova estimates were also analyzed using ANOVA with means comparisons between age classes to determine age at first reproductive maturity and reproductive pattern throughout the population's lifespan.

We used statistical model selection to determine what factors could be used as predictive variables for estimating reproductive potential and to elucidate spawn timing within the Boise River oriental weatherfish population. Our study locations are highly interconnected within the Boise River system, and fish may easily pass between collection sites so location specific effects are most likely minimized. Therefore, fish collected from all sample locations were pooled for analyses. Because collection time spanned 15 months, we compared ova per fish between calendar year for July (the only month that overlapped) using a Wilcoxan Rank Sum test. There was no significant effect of year on ova per fish ($Z = -.702$, $p > 0.5$) so fish were pooled between collection year. Three fish were excluded from analyses due to low sample size during winter months (Nov.-Apr.).

We modeled collection date (month), fish morphometric data (age, TL, weight, FCF) and rational between-variable interactions (month-TL, month-weight, month-FCF, age-TL, age-weight, age-FCF) as parameters against natural log ($\ln(x+1)$) transformed ova per fish for model selection using corrected Akaike's Information Criterion (AICc) and residual analysis (Cody and Smith 2006, Guy and Brown 2007).

The relevant parameters (TL, FCF, collection date and an interaction of FCF by month), as identified by the best fit model, were compiled into a predictive equation with which to estimate fecundity of female fish based on morphometric and phenological data. We estimated output values for fish of average length (sample-mean TL = 15 cm), using low (FCF = 2.0), average (sample median FCF = 5.9) and high (FCF = 8.4) body conditions by collection month. FCF was significantly correlated (Pearson's R = 0.62, $p > 0.05$) with ovary weight, and was chosen for this model as a non-lethal estimation of

body resources available to a given fish to be used for spawning (Anderson and Neuman 1996; Blanchard et al. 2003; Kennedy et al. 2008). Therefore, modeled estimates were plotted by month and FCF to elucidate spawn timing within our population.

Results

Sex Ratio and Population Description

Of the 237 dissected fish, 149 were confirmed as female and 84 as male by gonad examination (Table 2.1). Fin morphology confirmed 140 additional females and 175 additional males in the undissected fish. These totals produced a 1:1 sex ratio of 289 females and 259 males ($X^2 = 1.64$, p = 0.20) from the sampled areas. Thirty eight individuals were unidentifiable as to sex at the time of collection due to lack of pectoral fin differentiation (Urquhart and Koetsier 2011) or lack of gonad development and were deemed juveniles. Four of these fish were dissected and aged; these fish were included in age/growth analyses. The remaining 34 juvenile fish were utilized in analyses where age was not a factor. Due to trap mesh size, collections were highly biased toward larger (> 5 cm) fish. Juveniles were therefore underrepresented in this study. Female fish (n = 285) ranged in total length from 5.5 cm to 23.3 cm and in body weight from 0.7 g to 66.4 g; males (n = 255) ranged from 7.0 cm to 21.4 cm and from 1.4 g to 45.9 g respectively. Juveniles (n = 38) ranged from 2.8 cm to 10.0 cm TL and from 0.1 to 5.1 g in body weight (Table 2.1).

Age and Growth

Otolith sectioning identified five age classes in females, and seven age classes in males. Females ranged from 0-y to 4-y and males ranged from 0-y to 6-y. Of the four dissected juveniles, we were only able to locate and section the otoliths from the largest (TL = 7.6 cm) and this fish was aged at 0-y. Otoliths were not found in the other three juveniles (TL = 4.9 cm, 4.9 cm, 5.7 cm) and these were deemed 0-y fish. There was a significant effect of age on both TL and body weight ($F_{(6,214)}$ = 13.14, $p < 0.01$; $F_{(6, 214)}$ = 9.20, $p < 0.01$), such that older fish were larger and heavier than younger fish. However, means comparisons of TL between ages showed little size difference between years (Figure 2.3).

Sex also showed significant effects on TL ($F_{(1,214)}$ = 14.18, $p < 0.01$) and body weight ($F_{(1,214)}$ = 15.13, $p < 0.01$) with female fish being longer and heavier than males (Table 2.1). There was no interactive effect of age and sex on TL ($F_{(4,214)}$ = 2.30, $p =$ 0.06), although differences in TL between sexes were significant only for 1-y, 2-y, and 3-y fish (Figure 2.3). Males and females both reached the reported length at maturity (10 cm TL; Axelrod and Schultz 1955; Suzuki et al. 1985) within the first year, and fish showed continued growth throughout their lifespan.

Spawning and Fecundity

GSI ranged from 0.00 to 33.19 for females, and from 0.00 to 1.91 for males. Age and sex showed an interactive effect on GSI ($F_{(4,215)}$ = 2.60, $p < 0.05$) so sexes were separated for further analysis. The effect of age on GSI was significant for females ($F_{(4,141)}$ = 5.38, $p < 0.01$), such that gonadal tissue was proportionally larger in older female fish, but not so for males ($p = 0.28$). Female GSI showed a weak negative

relationship with collection date ($R^2 = 0.05$, $F_{(1,139)} = 8.68$, $p < 0.05$); GSI decreased in females throughout the calendar year, but not so for males ($p = 0.85$; Figure 2.4).

Estimated number of mature ova in female fish ranged from zero to 46,181 and fish in all age classes contained mature, vitellogenic ova (Figure 2.5). There was no significant effect of age ($p = 0.13$) or body condition ($p = 0.08$) on fecundity. However, both length ($R^2 = 0.06$, $F_{(1,66)} = 4.92$, $p < 0.05$) and body weight ($R^2 = 0.13$, $F_{(1,66)} = 13.18$, $p < 0.01$) independently showed significant positive effects on fecundity.

The top model of fecundity estimates (AICc = 549.99; Table 2.2) showed collection date, TL, FCF and an interaction between FCF and collection date as all having significant effect on fecundity. Applying the coefficients for each relevant variable we composed the following mathematical model for predicting fecundity of a given fish (i).

$$\ln(\text{ova}+1)_i = -6.5811 + TL_i (0.2276) + FCF_i (1.1356) + \beta_m + FCF_i (\beta_{FCF*Month})$$

Where β_m is the specific coefficient for each collection month and $\beta_{FCF*Month}$ is the specific coefficient for the interaction of FCF and collection month (Table 2.3).

Applying the model to fish of average size and body condition (mean TL = 15, median FCF = 5.9) from our collected samples, we identified a bi-seasonal pattern of fecundity, with maximum number of eggs available in late spring and late summer, interspersed with sharp declines of available eggs (presumably due to spawning) in June and October. This pattern of bi-seasonal fecundity held true for 75% of sampled fish. For example, when applied to body condition equal to first and third quartiles (FCF = 5.0, 6.4), drops in per fish egg availability were also displayed in June and October. Interestingly, at the minimum and maximum body conditions found in our samples, the

pattern appeared temporally compressed. At extremely poor body condition (FCF = 2.0), the first decrease in fecundity was delayed by one month, appearing in July, with the second still occurring in October. However, at extremely good body condition (FCF = 8.4), the first fecundity decrease took place in June, with the second advanced by one month, taking place in September (Figure 2.6).

Discussion

<u>Sex Ratio</u>

Within our study population, we found an approximate 1:1 sex ratio of sexually mature individuals (52.7% female, 47.3% male). Kubota (1961) found that sex ratio of oriental weatherfish populations within its native range varied as 51 - 98% female, and suggested that food rich environmental conditions favored development of females, while food poor environments favored males. These differences were attributed to dimorphism of growth patterns between sexes. Within our samples, females were larger than males in both TL and weight (Table 2.1) and would need an abundance of food to maintain their larger body size. Since sex ratio is not highly skewed toward either sex, and fits with Kubota's (1961) findings, oriental weatherfish in the Boise area may be relatively unstressed by food availability or temperature range (Fisher 1930; Trivers and Willard 1973; Nager et al. 1999). Instead, oriental weatherfish seem well adapted to the environmental conditions within this invaded habitat. Consequently, our findings may be typical of invasive populations of oriental weatherfish. Furthermore, similar patterns of

age, growth and fecundity to those reported here may be found within other successfully invaded regions.

<u>Age and Growth</u>

Examination of otoliths provided an age profile for the Boise River drainage oriental weatherfish population that spanned 7 years, with 1-y (34.4%) and 2-y (26.4%) fish representing the majority of the population. Fish grew to maturity within the first year (10 cm TL; Axelrod and Schultz 1955; Suzuki et al. 1985), and continued to grow throughout their lives. Growth slowed considerably after fish reached maturity and only small differences in body size existed between the early (0 - 3) and late (4 - 6) years (Figure 2.3). This pattern of rapid early growth followed by slow late growth makes non-lethal age estimation techniques for oriental weatherfish questionable at best. However, we are aware of no published age verification study of this fish and recognize the necessity of such validation. Given the well documented limitations of length frequency analysis or age/length predictive models (Jearld 1983; Devries and Frie 1996), and the invasive status of the oriental weatherfish, we suggest that additional ageing studies be carried out using techniques such as oxytetracycline (OTC) marking and Incremental Growth Analysis (Campana 2001). Notwithstanding, our application of visual otolith aging techniques indicated that oriental weatherfish in the Boise River drainage are capable of living in excess of 6 years.

Very little information is available regarding age and growth of native populations, however, Kubota (1961) reported on collections of 3,928 individuals from 1946, 1947, 1948, and 1954 in Yamaguchi prefecture, Japan. Mean body lengths ranged from 6.6 - 7.4 cm, with body weight means of 2.30 - 3.35 g. Approximately 90% of those

collected were under one year old, with very few individuals exceeding 15 cm. Lei and

Wang (1990) reported on growth of six age classes for both males and females under

aquaculture conditions, with highest growth during year one. Body sizes ranged from 6.0

- 23.8 cm in length and 1.8 - 103.2 g in weight. Suzuki (1983) experimented with 10 year

old captive raised female oriental weatherfish with body weights ranging from 27.4 - 40.6

g; TL was not reported for these fish. By comparison, our population means for body

length and weight were 12.64 cm/ 11.65 g for males and 14.93 cm/ 21.48 g for females

and approximately 20% of our collection was represented by year-zero fish. Our

sampling methods were admittedly biased against very small fish (< 5 cm). This bias may

be important because fish from our invasive population appeared to grow much larger

than those reported from the native range. Growth within our population appeared

comparable to that experienced in aquaculture conditions (Lei and Wang 1990) that are

specifically designed to promote fast and large growth for marketability as a food source.

However, if our sampling methods had equally sampled juvenile and small fish, we may

have seen growth patterns equivalent to those found in the native range.

Sexual Maturity

Sexual maturity in oriental weatherfish is typically reported as being reached at 10

cm TL, (Sterba 1963; Wydoski and Whitney 2003). However, Kubota (1961) reported an

expansion and change in ovary shape upon fish reaching 7 - 8 cm in length, indicating

sexual maturity may have been reached at a much smaller size. Lei and Wang (1990)

found early-stage gonad development in both 0-y and 1-y fish ranging from 7 - 10 cm,

but the smallest fecund female was 11.1 cm in length. Fish in our samples often reached

10 cm TL prior to reaching one year of age. Mean TL for 0-y fish in our study was 11.14

cm for females and 11.46 cm for males (Figure 2.3). We found three 0-y fish (13.0, 14.7 and 18.0 cm TL) that were fecund; however, the smallest fish in which we found vitellogenic ova was 12.6 cm TL and this individual was 2 years old. Body size appears to be a better indication of sexual maturity than age; however, empirical studies specifically targeting these data are sparse. Those that do exist are in disagreement as to the specific size at which maturity is reached. However, in each of these studies, size of sexual maturity was often reached prior to the completion of the first year of life and females were capable of producing viable ova by the end of year zero. Fish within the Boise River population appeared to follow the same age and sexual maturity patterns reported for populations within their native range.

Fecundity

Oriental weatherfish are capable of very high reproductive output beginning at an early age. Lei and Wang (1990) found 2,000 – 46,000 ova per fish collected within their native range, and this characteristic is retained within the Boise River population as well. Females in our samples showed fecundity throughout all age classes (Figure 2.5), with the largest fish containing upwards of 40,000 vitellogenic ova potentially available during any given spawning event. Number of ova per fish is related to body mass, but it is also highly variable. Suzuki (1976) reported that body mass was important to fecundity, but only for fish above 15.2 g.

Suzuki and Yamaguchi (1977) suggested that maturation of the ovary and by extension, ripening of ova may depend on temperature. They found that at 15° and 20° C, less than 20% of sampled oriental weatherfish spawned, when injected with Human Chorionic Gonadotropin (HGC). By contrast, 79% of oriental weatherfish reared at 25°

and 30° C spawned within 14 hours of injection. This temperature dependent effect held for induced out-of-season spawning as well, indicating that temperature may play a key role in fecundity in oriental weatherfish.

At our collection sites, water temperature ranged from 0 - 38° C, with one location showing daily temperature variation of up to 10° C. These highly variable temperatures may play a significant role in explaining the variability in fecundity seen throughout the Boise River population. However, we also found young of the year at all of these collection sites. Consequently, we cannot discount the effect of temperature on reproduction, but from our data we infer that oriental weatherfish can adapt and reproduce within a wide range of temperatures. For instance, Suzuki and Yamaguchi (1977) successfully induced spawning in fish collected during the winter, from waters at 5° C, after brief exposure (10 days) to water temperatures of 25° C. Regardless of the specific effects that water temperature may have on oriental weatherfish fecundity, the Boise River population has retained, within these invaded habitats, the same high fecundity as found within its native regions.

Breeding Season and Spawn Frequency

High variability of GSI found throughout the summer months (Figure 2.4), coupled with highly variable ova counts throughout the same time period (Figure 2.5) indicates that oriental weatherfish are most likely asynchronous or multiple spawners (Jons and Miranda 1997; Brewer et al. 2008; Helfman 2009). Our sample population utilized the spring through autumn months as a protracted spawning season. This same seasonality was reported by Saitoh et al. (1988) and Fujimoto et al. (2008) while studying migration patterns of oriental weatherfish moving from streams, to spawn in rice paddy

fields, throughout Japan. Suzuki (1976) reported that ovaries of fully mature, fecund females contained ova of various sizes, indicating asynchronous ripening of ova throughout the breeding season, and the possibility of multiple spawning events.

To investigate the plausibility and timing of multiple spawning events, we used AICc statistical analysis to create a model utilizing morphometric, temporal and fecundity data from our sampled population. When we applied the resulting mathematical model to fish of average size and body condition (mean TL = 15 cm, median FCF = 5.9) from the Boise River population, a bi-seasonal pattern of fecundity emerged. Oriental weatherfish reached maximum fecundity in early spring (May) and deposit eggs during late spring (June). This spawning event was followed by approximately two months of ova regeneration then additional spawning in late fall (October; Figure 2.6). The timing of these spawning events was highly dependent upon parental body condition. In fish with very good body condition, ova regeneration appeared to happen very rapidly and the second spawning event took place a month earlier than in fish with average body condition. When body condition was poor, possibly due to food limitation or habitat unsuitability (Anderson and Neuman 1996), the first spawning event appeared delayed until later in the spring. However, this delayed event was followed by rapid ova regeneration and a second spawning event in October, coinciding with the same second spawning event in fish with average body condition. Overall, this pattern of egg availability indicates that a sexually mature, average sized fish may participate in two spawning events per season; the timing of which appears contingent upon body condition.

Furthermore, variability in otolith banding patterns suggests that young emerge during both Spring and Autumn. When a fish emerges just prior to a fast growth period, the first band encircling the larval focus appears translucent (but see Jearld 1983; Hales and Belk 1992; Anderson and Neuman 1996). When the fish emerges just prior to a period of slow growth, the banding pattern may be opposite; i.e., the first band surrounding the larval focus appears opaque. Additionally, when a fish emerges mid-season, it may experience a truncated season of fast growth, leading to a thin translucent band followed by a relatively thicker, opaque band. We saw a mixture of these banding patterns throughout our sample sites, and coupled with our fecundity model, we concluded that oriental weatherfish in the Boise River system are spawning twice per summer season.

This seasonal spawning may take place as two discrete, coordinated events, shared by the entire population, or as two single spawning events carried out by different fish within the same population. Either scenario is possible considering the high variability of GSI and ova counts found within our samples. It is probable that spawning events are shared within the entire population given the retained coordination of at least one spawning event per year at extremely high and low body condition. Body condition and food availability has been shown to affect spawn timing in several fish species (Blanchard et al. 2003; Kennedy et al. 2008). Where limited resources cause growth deficiency (low FCF) it may benefit the fish to delay spawning until sufficient body mass has accumulated, especially if mate selection is competitive or driven by body condition (Cogliati et al. 2010). If oriental weatherfish are batch spawners, late developed ova may be available for a second spawning event, coordinated with the rest of the population.

Additionally, a fish higher in body condition may share a coordinated spawning event early in the season, and due to increased energy resources and relative body size (Pecquerie et al. 2009) produce a second offspring batch earlier than its cohorts (Slotte et al. 2000). These offspring may gain a competitive advantage through increased growth in the first season. Suzuki (1976, 1983) found ova at multiple stages of development throughout the year, and under laboratory conditions successfully induced monthly spawning in captive oriental weatherfish for 13 consecutive months. While duplication of this extreme example seems unlikely under natural conditions, our model coupled with Suzuki's findings suggests that oriental weatherfish within invaded regions have retained the ability to spawn multiple times during each 4 - 5 month summer breeding season.

Successful invasive fish species have been well characterized as possessing certain life history traits. Among these are long lifespan, early maturation, high fecundity, and broad environmental tolerance (Townsend 1996; Marchetti et al. 2004; Vila-Gispert et al. 2005). Fish in our study showed all of these characteristics. Our samples displayed life spans of up 7 years, with rapid growth to sexual maturity, and reproduction within the first year of life. At that time fish were capable of very high reproductive output, and our model predicted multiple spawning over a protracted mating season. We collected fish from water bodies with widely ranging water temperatures (0° - 38° C) and as much as 10° C daily temperature variation. Oriental weatherfish can survive inhospitable environmental variables such as hypoxic water conditions and aerial exposure through surface air breathing and cuticular volatilization of nitrogenous wastes (Chew et al. 2001; Tsui et al. 2002; Ip et al. 2004; McNeal and Close 2007). They can survive months of habitat drying, and resist desiccation by burrowing into the substrate; waiting out these

conditions in a small excavated chamber, several centimeters below the ground surface (Koetsier and Urquhart 2012).

Within their native and invaded ranges, oriental weatherfish utilize highly disturbed habitats (irrigation canals, ponds, rice paddies; Saitoh et al. 1988; Fujimoto et al. 2008; Urquhart and Koetsier 2011). Many of these relatively new ecosystems differ from surrounding landscapes and competition or predatory threat from native communities may be limited. Worldwide, oriental weatherfish may be successfully establishing new populations due to release from competition with native range co-occurring species (*Carassius carassius*; Kubota 1961; Wolfe 2002), or due to losses of native species that would otherwise compete for soft muddy substrates (*Lampetra tridentata* ammocoetes; Stone and Barndt 2005). Continuous introduction and intense propagule pressure (Kolar and Lodge 2001; Lockwood et al. 2005) through household aquarium and ornamental pet releases (Chang et al. 2009; Strecker 2011) coupled with its ability to adapt to a wide range of environmental factors (Koetsier and Urquhart 2011) may also play significant roles. However, very little beyond mere species accounts have been published of invasive oriental weatherfish populations, and there exists a dire need for further ecological research.

Given its current status as a relatively unknown but widespread invader, we suggest that the oriental weatherfish become a model invasive species on which further study of ecological and invasive species theory may be based. The air-breathing capabilities and unique physiological (McMahon and Burggren 1987; Chew et al. 2001; Ip et al. 2004) and reproductive characteristics of this fish (polyploidy, unreduced gametes, hybridization; Arias-Rodriguez et al. 2007; Morishima et al. 2008) offer broad subjects of

research into genetic questions of evolution and speciation. Adding to this the potential for pathogen introduction and interaction with economically valuable species; clearly, the oriental weatherfish should be viewed as an invasive species of import with as yet unknown ecological impacts.

Acknowledgments

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Table 2.1. Descriptive statistics of oriental weatherfish collected and sampled from water bodies connected with the Boise River, Boise, Idaho, USA. A significant difference exists in both TL and body mass ($F_{2,574} = 144.8$, $p < 0.001$; $F_{2,574} = 81.6$, $p < 0.001$) between groups (Male, Female, Juvenile).

		Total Length (cm)			Weight (g)		
	N	Mean	SE	Range	Mean	SE	Range
Males	255	12.64	0.16	7.0 - 21.4	11.65	0.47	1.40 - 45.90
Females	285	14.93	0.2	5.5 - 23.3	21.48	0.86	0.70 - 66.40
Juveniles	37	6.46	0.33	2.8 - 10.0	1.75	0.24	0.14 - 5.10

Table 2.2. Statistical models explaining fecundity estimates during the spawning season (June - October) of oriental weatherfish as determined by AICc values. Model parameters included collection date (month), fish morphometric data (age, TL, weight, FCF) and rational between-variable interactions against natural log transformed (ln(x+1)) ova/fish.

Rank	Model Parameters	AICc	ΔAICc	K	Weight	LL
1	length collected bc collected*bc	549.99	0.00	16	0.9888	-256.48
2	weight collected	562.23	12.24	10	0.0022	-270.15
3	length collected bc collected*length	562.61	12.62	16	0.0018	-262.79
4	weight length collected	562.66	12.67	11	0.0018	-269.16
5	weight collected bc	562.73	12.74	11	0.0017	-269.20
6	length collected bc	563.75	13.76	11	0.0010	-269.71
7	weight length collected bc	564.95	14.96	12	0.0006	-269.08
8	collected bc	565.26	15.27	10	0.0005	-271.67
9	length collected bc weight collected*weight	565.50	15.51	17	0.0004	-262.89
10	age weight length collected	566.81	16.82	15	0.0002	-266.21
11	age weight collected bc	567.13	17.14	15	0.0002	-266.36
12	age collected bc	567.24	17.25	14	0.0002	-267.71
13	age weight collected	567.57	17.58	14	0.0002	-267.88
14	length collected	567.73	17.74	10	0.0001	-272.90
15	age length collected bc	568.01	18.02	15	0.0001	-266.80
16	collected	568.68	18.69	9	0.0001	-274.56
17	age weight length collected bc	569.29	19.30	16	0.0001	-266.13
18	weight length	571.40	21.41	4	0.0000	-281.53
19	weight bc	571.74	21.75	4	0.0000	-281.70
20	age collected	571.76	21.77	13	0.0000	-271.24
21	age weight length	572.05	22.06	8	0.0000	-277.41
22	weight	572.73	22.74	3	0.0000	-283.27
23	age length collected	573.04	23.04	14	0.0000	-270.61
24	age weight bc	573.14	23.14	8	0.0000	-277.95
25	weight length bc	573.39	23.40	5	0.0000	-281.44
26	age weight length bc	574.33	24.33	9	0.0000	-277.38
27	age weight	574.53	24.54	7	0.0000	-279.79
28	length collected bc age age*length	574.81	24.82	19	0.0000	-264.79
29	length bc	575.24	25.25	4	0.0000	-283.46
30	length collected bc age age*bc	575.87	25.88	19	0.0000	-265.32
31	age length bc	577.13	27.14	8	0.0000	-279.95
32	length collected bc age weight age*weight	577.31	27.32	20	0.0000	-264.62
33	length collected bc age collected*age	580.70	30.71	31	0.0000	-248.68
34	age bc	586.28	36.29	7	0.0000	-285.66
35	bc	588.73	38.74	3	0.0000	-291.27
36	length	592.01	42.01	3	0.0000	-292.90
37	age length	595.90	45.91	7	0.0000	-290.47

| 38 | null | | 607.38 | 57.39 | 2 | 0.0000 | -301.64 |
| 39 | age | | 607.45 | 57.46 | 6 | 0.0000 | -297.37 |

Table 2.3. Parameters and coefficients used to create a mathematical model for estimating fecundity and spawn timing of oriental weatherfish from collection sites connected with the Boise River, Boise, Idaho, USA. Model parameters and interactions were selected based on AICc model selection and residual analysis.

Parameter	Month	Coefficient	SE	DF	t Value	Pr > |t|
Intercept		-6.5811	3.9695	109	-1.66	0.1002
TL		0.2276	0.1033	109	2.20	0.0298
FCF		1.1356	0.5649	109	2.01	0.0469
Month	May	6.8719	8.6318	109	0.80	0.4277
Month	Jun	13.8603	5.1524	109	2.69	0.0083
Month	Jul	0.9451	5.2784	109	0.18	0.8582
Month	Aug	18.3338	5.1079	109	3.59	0.0005
Month	Sep	3.9250	3.5831	109	1.10	0.2757
Month	Oct	0.0000	.	.	.	.
FCF*Month	May	-0.4224	1.3910	109	-0.30	0.7619
FCF*Month	Jun	-2.7194	1.0336	109	-2.63	0.0097
FCF*Month	Jul	0.2672	0.8229	109	0.32	0.7461
FCF*Month	Aug	-2.5543	0.8002	109	-3.19	0.0018
FCF*Month	Sep	-0.4635	0.6053	109	-0.77	0.4455
FCF*Month	Oct	0.0000	.	.	.	.
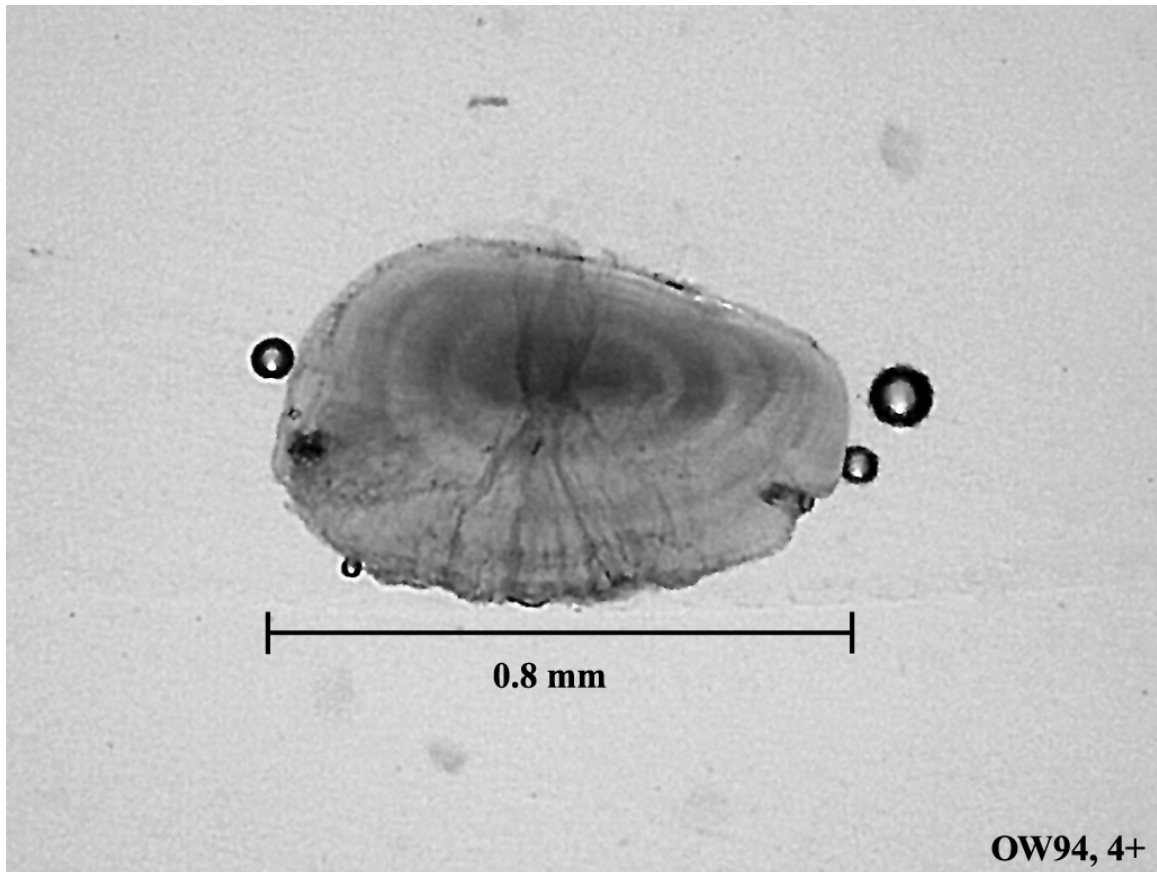
Figure 2.1. Lapillus otolith section of oriental weatherfish, showing typical banding pattern consisting of concentric translucent and opaque bands. Otolith was taken from a male fish, 14.3 cm TL. Paired bands of opaque/translucent regions were counted as 1 year of growth such that this fish was classified as a 4-y fish.
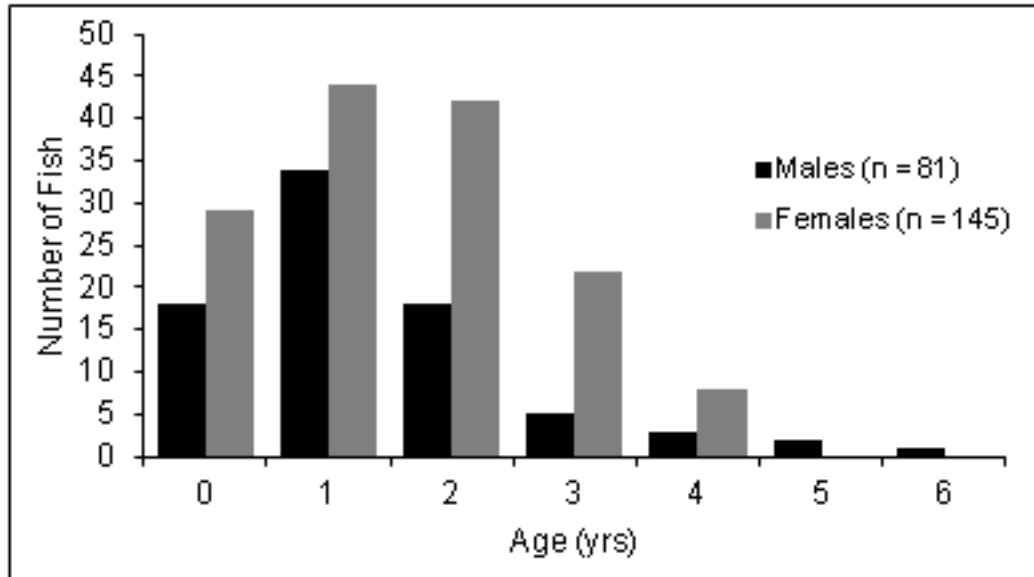
Figure 2.2. Age frequency of oriental weatherfish as determined by sectioned otolith ageing. Males show seven age classes and females show five age classes. Age was determined by counting opaque and translucent banding pairs encircling the larval focus of the lapillus otolith from dissected fish.
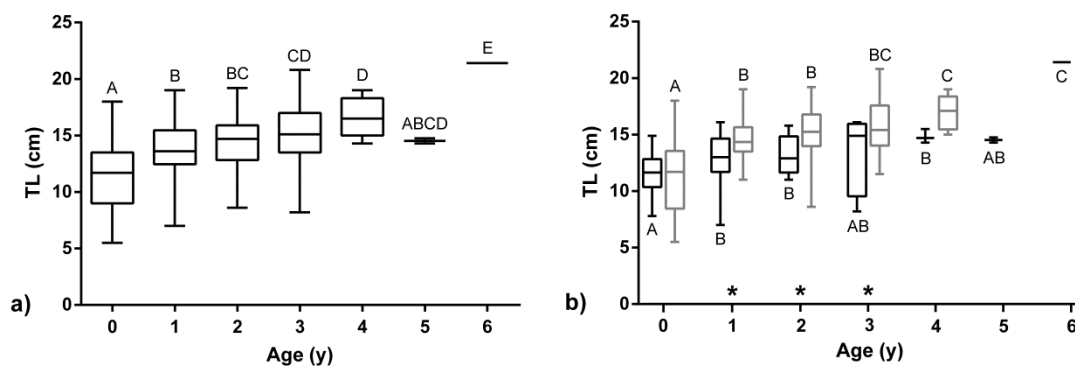
Figure 2.3. a) Total length, by age in a sample of oriental weatherfish collected from water bodies connected with the Boise River, Idaho, USA. Age had a week positive effect on fish length ($R^2 = 0.25$, $F_{(1,224)} = 74.61$, $p < 0.01$) and fish showed continued growth throughout all ages. Means comparisons of TL revealed little difference between age groups past 1-y. b) When analyzed separately by sex, females (grey boxplots) were generally larger than males (black boxplots), however length differed significantly between sexes only in ages 1-y, 2-y, and 3-y fish (indicated with *). Means comparisons between ages for females are displayed above the corresponding boxplots; those for males are displayed below. Both male and female weatherfish reached the reported age of maturity (10 cm TL) within the first year of life.
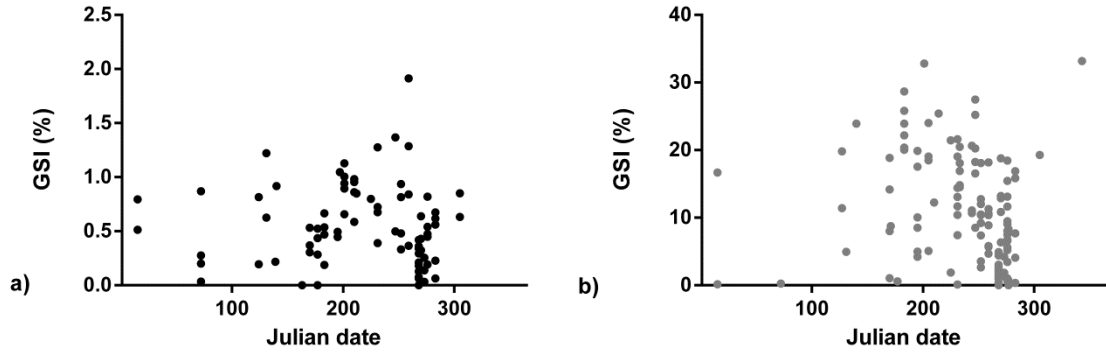
Figure 2.4. GSI of a) male, and b) female weatherfish collected from water bodies connected with the Boise River, Idaho, USA. Both males and females showed high variability in GSI throughout the spawning season (May - Oct) and females showed a weak negative relationship between collection date and GSI ($R^2 = 0.05$, $F(1,139) = 8.68$, $p < 0.05$) such that GSI decreased throughout the calendar year.
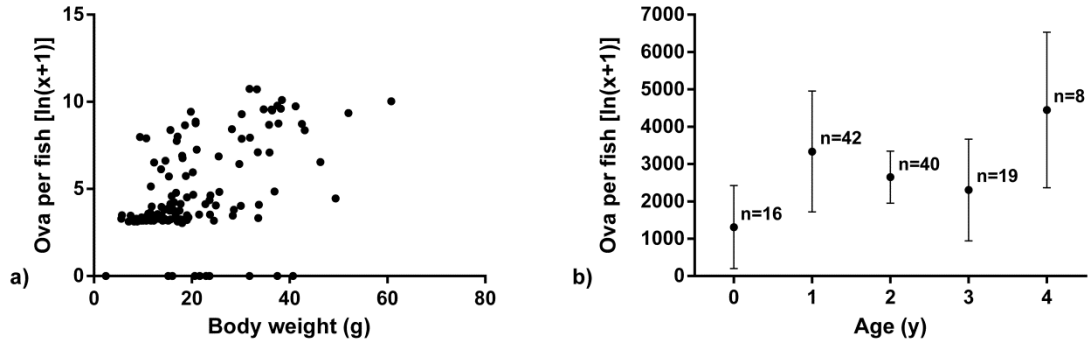
Figure 2.5. Fecundity of oriental weatherfish collected from water bodies connected with the Boise River, Idaho, USA. a) As female body mass increased, there was a corresponding increase in ova produced (Adj. R^2 = 0.12, $F_{(1,66)}$ = 13.18, p < 0.01) with the most fecund fish carrying an excess of 40,000 vitelogenic ova at time of collection (n = 127). b) Oriental weatherfish of all age classes carried vitelogenic ova at time of collection (n = 125).
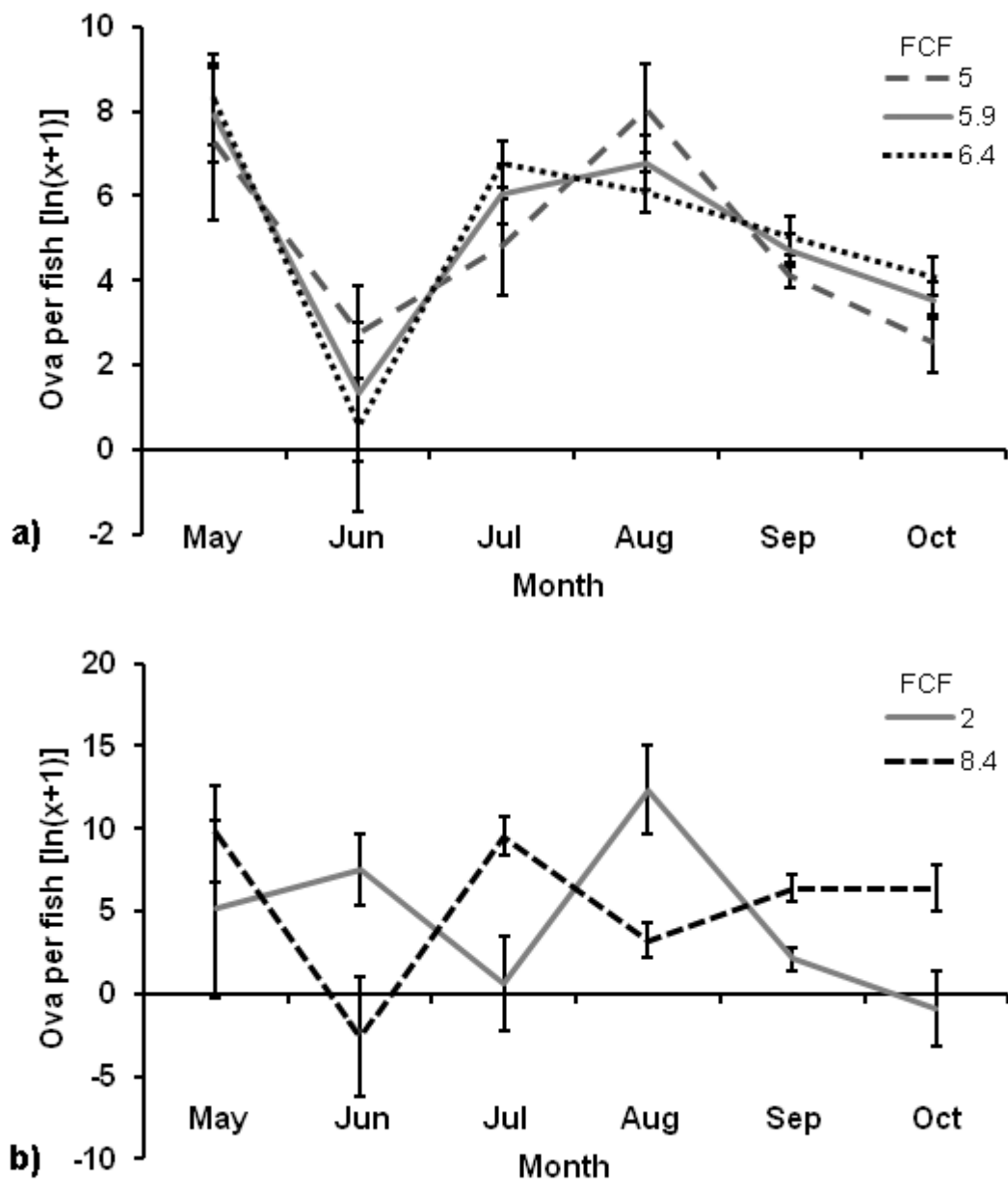
Figure 2.6. Estimated ova produced by a 15 cm TL oriental weatherfish at differing body conditions as predicted by AICc model selection and residual analysis. Fish at median, first and third quartiles display a bimodal fecundity cycle, indicating two spawning events within the population, each followed by a period of ova production. The timing of spawning events appears contingent upon body condition; extremely low and high body conditions (FCF = 2.0, 8.4) display a temporal shift in predicted spawn timing.

CHAPTER THREE: DIET OF A CRYPTIC BUT WIDESPREAD INVADER, THE

ORIENTAL WEATHERFISH (*Misgurnus anguillicaudatus*) IN IDAHO, USA

Abstract

The oriental weatherfish (*Misgurnus anguillicaudatus*) is invasive to 13 states in the USA and at least nine other countries. Although widespread, very little is known regarding the ecology of this cryptic fish. As part of a comprehensive study into the fish's life history, we collected 237 individuals from water bodies connected to the Boise River (USA), to determine the diet of the fish within this invasive population. Fish were collected using baited minnow traps. We dissected and examined the stomach contents and found most of the stomachs (158 of 237) were empty and 42 contained only the trap bait. However, 37 contained natural food items taken from the fish's environment. Gravimetric analysis, frequency of occurrence and mean % by number indicated benthic invertebrates as the primary diet component, with chironomid larvae as the most numerous prey eaten. Graphical analysis of prey-specific abundance also indicated that the oriental weatherfish feed on a generalized diet, of which detritus is likely an important part. An omnivorous diet may, in part, explain the fish's ability to expand its invasive range quickly and successfully. Our findings add to a growing list of traits indicating that the oriental weatherfish fits the profile of a highly successful invader and as such, should be a target species of further ecological research.

Introduction

Global aquatic biodiversity is being threatened by invasive species (Olden et al. 2004; Villeger et al. 2011). In the United States alone, an estimated 138 non-native fish species have been introduced (Pimentel et al. 2005). Many of these have become established and are now considered a permanent part of the aquatic faunal communities (Moyle and Light 1996; Mooney and Cleland 2001). These new residents often disrupt existing community structure through competition, predation, habitat modification and pathogen introduction, and may lead to loss of native biota (Mills et al. 1994; Dextrase and Mandrake 2006; Vitule et al. 2009). Most studies have focused on iconic invasive organisms, such as introduced sport fish (e.g., Townsend 1996) or those that have shown high economic impacts (e.g., zebra mussel, *Dreissinea polymorpha,* Ricciardi 2003). However, many less charismatic species have been introduced, about which very little is known. These cryptic species may be increasing their range with as yet unknown ecological impacts. The oriental weatherfish (*Misgurnus anguillicaudatus*) is one such invader.

The oriental weatherfish is native to eastern Asia, with longitudinal range spanning from India to Viet Nam and latitudinal range as far north as southern Russia. In Japan and other native countries, the fish is used as live bait for sport fishing, grown for sale as a food product, and exported as an exotic aquarium pet fish (Wang et al. 2008). International commerce associated with the aquarium trade may have contributed to the fish's rapid range expansion (Chang et al. 2009, Strecker et al. 2011). Currently, the oriental weatherfish is found in ten countries outside of its native range including the Philippines, Turkmenistan, Italy, and Spain (Freyoff and Korte 2005, Simon et al. 2006,

Franch et al. 2008), Germany (Meyer and Hinrichs 2000), and Australia (Keller and Lake 2007). Range expansion of the fish appears to be ongoing and most recently, populations of oriental weatherfish have been described in the Netherlands (van Kessel et al. 2013) and Brazil (Abilhoa et al. 2013). Within North America, the fish is established in Mexico (Contreras and Escalante 1984) and 13 of the United States (Fuller et al. 1999, Tabor et al. 2001, Ashton and Ciccotto 2010). In Idaho, the first reported oriental weatherfish was collected near the town of Eagle, in 1951 (Courtenay et al. 1987). Since that time, a self-sustaining population has been thriving within irrigation canal and pond systems with seasonal connection to the lower Boise River (Urquhart and Koetsier 2011).

Studies that examine the impacts of the invasive oriental weatherfish on native fish communities are lacking. However, potential threats to native species have been identified in a few cases. In Australia, the fish is banned from importation due in part to concerns over decreases in native fish populations where the oriental weatherfish has established (Burchmore et al. 1990, Arthington and Bludhorn 1995). Additional concerns stem from the fish's dietary overlap with native species (Lintermans 2002). In Germany, an endangered native congener (*M. fossilis*) may be susceptible to further habitat loss due to its invasive sister species (Meyer and Hinrichs 2000, Freyhof and Korte 2005). Further impacts may include competition with native species for invertebrate prey (Keller and Lake 2007) and direct predation of native fish eggs and larvae (Logan et al. 1996).

Currently, the invaded range of the oriental weatherfish includes disparate locations around the globe, and each invaded habitat may present the fish with specific resource availability. As a result, the oriental weatherfish may adapt its diet to locally available food sources, a strategy employed by other invasive fishes (Harms and

Turingan 2012, Kornis et al. 2012, Borcherding et al. 2013). Consequently, determination

of diet is necessary to elucidate the ecological role (and the potential threats to native

fishes) that the oriental weatherfish holds within each of its invaded regions. To this end,

as part of a larger study designed to define several life history components of the fish, we

conducted a diet analysis of oriental weatherfish collected from canals and ponds

connected to the Boise River, (USA).

Methods

From May- 2008 to July- 2009, we collected oriental weatherfish from six

water bodies seasonally connected to the Boise River (for collection sites, GPS

coordinates, and voucher specimens see Urquhart and Koetsier 2011). We placed

minnow traps in suitable sites based on published descriptions of oriental weatherfish

habitat (Allen 1984, Tabor et al. 2001, Freyhof and Korte 2005) and Idaho Department of

Fish and Game collection records (IDFG personnel, personal communication). Traps

were made of vinyl-coated steel mesh (4 mm mesh size), approximately cylindrical and

measured 42 cm x 22 cm. Each end had a 2.5 cm opening at the apex of an inverted cone.

Each trap was baited with approximately 30 g of dry commercial dog food, submerged

upon the substrate at each site and marked with a small floating buoy. Traps were placed

singly at each location and were moved or relocated to new collection sites based on site

accessibility and seasonal dewatering. Water depth at each trap opening varied by

location and ranged from 10 cm to approximately 1.5 meters.

We checked traps every 4 to 10 days during the sampling period. All

captured oriental weatherfish were moved to the Boise State University, Aquatic Ecology

Laboratory. A subsample of collected fish was hand-netted from collection buckets without regard to sex or size. These fish were euthanized using a dilution of tricaine methanosulfonate (MS222) in freshwater and dissected immediately or frozen at -4°C and later, thawed for dissection. Of the 586 collected fish, 237 were euthanized and dissected; all others were housed in laboratory aquaria for later, experimental use. Euthanized fish were sexed following Urquhart and Koetsier (2011), wet weighed (0.1 g), and measured for total length (TL). Fish were chosen for dissection based on time available for dissection, and number of fish awaiting dissection without regard for sex, size, collection date, or collection location. Each fish was incised from the vent forward through the pectoral girdle, and the alimentary canal was removed by excision at the vent and the foregut, just forward of the pectoral girdle. Heart, liver and extraneous connective tissues were removed and discarded. Each complete digestive tract was preserved in 70% ethanol until used for diet analysis.

We dissected each stomach and emptied the contents into a small glass bowl. Intestinal contents were not enumerated in this study due to level of digestion making identification of contents impossible. Stomach contents were examined under a dissection scope at 10x – 60x power (Zeiss Stemi 2000-C) and each item was classified as one of three broad categories; invertebrates, vegetation, or inorganic substrate. When possible, invertebrates were identified to order or family then enumerated. Stomach content samples (separated into the three categories) were placed into aluminum pans and oven-dried for 24 to 48 hours at 105°C (Bowen 1996). From each dry-weight sample, we calculated percent composition by mass of each category per fish. Within the invertebrate category we divided each sample into individual taxa and calculated frequency of

occurrence based on the number of stomachs in which each was found. For these calculations, disarticulated pieces of invertebrates were grouped together regardless of taxa or number of parts. We also included vegetation and inorganic substrate in frequency of occurrence calculations.

To compare the relative abundance between individual food items, we calculated mean percent-composition-by-number (%N_i: mean % by number) using the formula

$$\%N_i = \frac{\sum_{j=1}^{n_j} N_{ij}}{\sum_{i=1}^{n_i}\sum_{j=1}^{n_j} N_{ij}}$$ where N_{ij} is the number of prey taxon (i) in the stomach of a fish (j),

n_i is the number of prey taxa, and n_j is the number of stomachs containing food (Tirasin and Jørgensen 1999). For these calculations, disarticulated arthropod pieces and soil components were assigned the value of a single organism regardless of the total amount found within a stomach. We also calculated prey-specific abundance (P_i) using the

formula $P_i = \frac{\sum S_i}{\sum S_{ti}}$, where S_i is the number of prey i found in a given stomach, and S_{ti} is

the total number of prey in fish that contained prey i. We then plotted prey-specific abundance against frequency of occurrence to determine the feeding behavior and relative importance of each prey item following Amundsen et al. (1996). In this modification of Costello (1990), grouping of prey items along either axis or along the diagonal indicates the level of specialization or generalization of feeding within the population. This method also allows comparison of individual food items such that higher P_i values indicate higher importance of dietary components.

Results

Dissected fish consisted of 84 males, 149 females and four juvenile fish. Body size ranged from 4.9 cm to 23.3 cm TL (mean = 13.8 cm, SE = 0.2 cm) and from 0.6 g to 60.8 g (mean = 16.4 g, SE = 0.7 g) wet weight. Of the 237 dissected fish, 158 individuals had empty stomachs, and 42 stomachs contained only the food bait. The bait was easily recognizable as a yellow, oily, non-cellular mass in the stomachs. The remaining 37 individuals were comprised of 18 males, 18 females and one juvenile, ranging in length from 7.0 cm to 21.4 cm (mean = 15.2 cm, SE = 0.5 cm) and ranging in wet weight from 2.0 g to 52.0 g (mean = 22.7 g, SE = 2.1 g). Within the stomachs of these individuals, invertebrates made up 78.4% of ingested material by mass, followed by vegetation (15.0%) and inorganic substrate (silt, sand, and pebbles, diameter < 3 mm; 7.2%). Frequency analysis showed that invertebrate materials were present in 40.5% of the stomachs, followed (in frequency) by chironomid larvae (29.7%), vegetation (24.3%), odonate naiads and adults (21.6%) and small freshwater clams (18.9%). Additional contents consisted of snails, small crustaceans (amphipods and *Daphnia*), ants, a single small annelid worm, and aquatic hemipteran insects (Table 3.1). Vegetation was unidentifiable to taxon and primarily consisted of small (less than 3 mm in length) pieces of fibrous plant matter. Soils were present in 18.9% of stomachs.

Chironomid larvae were present in the greatest abundance of any given prey item (mean = 25%, SE = 13.9), followed in number by freshwater clams (mean = 15.2%, SE = 7.9) and disarticulated pieces of arthropods (mean = 13.2%, SE = 3.8); however no single food item was eaten in high relative abundance (Figure 3.1). Plots of prey-specific abundance of individual prey showed clustering along the diagonal, with most prey items

located in the lower left quadrant when plotted against frequency of occurrence per fish (Figure 3.2). This grouping is indicative of a generalist diet expressed by the population as a whole, with most fish feeding on prey items of low relative importance (Costello 1990, Amundsen et al.1996). In concordance with abundance data, chironomid larvae again represent the most important invertebrate prey species ingested by the fish, followed in importance by disarticulated arthropod pieces.

Discussion

Oriental weatherfish associated with the lower Boise River ate a generalized diet, feeding primarily on a variety of benthic macroinvertebrates. However, the most frequently eaten food items were chitinous pieces of invertebrate exoskeletons. Several of these parts contained soft tissue, suggesting that they were pieces of prey taken live by the fish (Keller and Lake 2007). Conversely, some of these disarticulated pieces appeared to be the molted exuviate of emerging insects. Detritus may therefore also be an important part of the oriental weatherfish's diet, and nutrients or important organic components (e.g., carbon, nitrogen) might be obtained through digestion of this material (Helfman et al. 2009). However, a small proportion of the stomachs also contained some inorganic substrate (sand, silt, small pebbles) suggesting that detritus, along with substrate materials may have been accidentally ingested. Oriental weatherfish use their oral barbels to locate prey by tactile and chemoreception (Watanabe and Hidaka 1983). Once located, the fish suck up invertebrates from the substrate, possibly along with other benthic material. Thus, exuviate and substrate particles in the gut may be the unintended result of the fish's feeding behavior.

Chironomid larvae were the second most frequently eaten prey item and were eaten in the largest proportion. Consequently they represented the most important individual prey item (Amundsen et al. 1996). Most of these larvae were found intact and easily identified by the attached head capsules, which may indicate live prey consumption. However, we could not determine if oriental weatherfish preferred this prey item or if chironomids were the most abundant invertebrate in the habitat. This reveals a disadvantage in using passive trapping methods in the eventual analysis of gut contents. Oriental weatherfish are difficult to collect by electrofishing or active net seining due to their behavior of burrowing into the substrate and their relative insensitivity to electrical current (Simon et al. 2006, Koetsier, personal observation). As a result, passive, baited minnow traps were used in this study. Fish may have been in the traps for several days, during which time they may have continued to feed opportunistically on non-preferable food items along the bottom of the trap. As a result, stomach contents may be a result of trap location rather than fish preference. Additionally, loss of easily digestible material during entrapment is also a concern (Bowen 1996). Although our analyses indicated a benthic invertebrate diet, other soft bodied organisms, algal cells, phytoplankton and other microscopic foods may have been digested while the fish were in the traps, leaving behind only the harder to digest invertebrate prey.

Because of the high number of empty stomachs we encountered during this study, we undertook a brief examination to observe gut retention time of oriental weatherfish. Small, freshwater clams (Sphaeriidae, less than 4 mm diameter), New Zealand mudsnails (*Potamopyrgus antipodarum*) and commercial fish food pellets were fed to captive oriental weatherfish (n = 9). Fish were later dissected and all food items were found to

have been passed from the stomachs within 6 to 24 hours of feeding (Urquhart and Daw unpubl. data). This limited retention time, coupled with the long entrapment periods undoubtedly lead to the high proportion of empty stomachs observed in this study.

Within its native range, the oriental weatherfish is carnivorous when small, feeding mainly on zooplankton (Kubota 1961) and small, benthic invertebrates such as emphermeropteran, trichopteran and chironomid larvae (Katano et al. 2003). The fish tends to undergo an ontological diet shift at approximately 10 - 13 cm total length, switching to an herbivorous diet (Kubota 1961). In our study, fish that contained natural food items ranged from 7.0 cm to 21.4 cm TL (mean = 15.2 cm) and fed primarily on benthic invertebrates, with only nine stomachs (of 37) containing vegetation. Our data thus suggest that oriental weatherfish, within the Boise River system, may have a much longer carnivorous diet period (and at a larger size) than found in their native range. These findings are in keeping with those from the few other diet studies conducted on invasive oriental weatherfish populations. In Australia (Burchmore et al. 1990, Lintermans et al. 1990) and Washington State, USA (Tabor et al. 2001), benthic macroinvertebrates were the primary prey and fish were feeding on this carnivorous diet at sizes similar to those in our study. Thus, oriental weatherfish may be adapting to invaded habitats through diet switch or delay of the characteristic ontological change seen within the fish's native range.

As a relatively unknown but widespread invasive species, the oriental weatherfish may pose several threats to other aquatic fauna. Under experimental conditions, oriental weatherfish caused significant decreases in invertebrate populations (Keller and Lake 2007). Many native and sport fishes (e.g., Pacific salmon and steelhead,

Oncorhyncus spp.) rely on invertebrates as a primary food source during development (Bellmore et al. 2013). Competition for important invertebrate prey may be a substantial factor where these fishes are endangered or actively targeted for recovery efforts. Habitat modification, including increased water turbidity (Keller and Lake 2007), and direct predation of larvae or eggs of native fish (Logan et al. 1996) have also caused concerns where oriental weatherfish share habitat with native fishes. Additionally, at least one monogaean parasite is known to have been introduced through release of imported oriental weatherfish (Dove and Ernst 1998), and the fish is also known to carry the LV1 Birnavirus (a close relative of Infectious Pancreatic Necrosis Virus; Wolf 1988, Lintermans et al. 1990).

Continuous propagule pressure (Lockwood et al. 2005) through the release of unwanted aquarium pets (Chang et al. 2009, Strecker et al. 2011) increases the likelihood of establishment of new oriental weatherfish populations. Simultaneously, high fecundity and multiple spawning events (Suzuki 1983) may aid in expansion of the fish's current invaded range. Furthermore, seemingly unfavorable environmental conditions may do little to limit the invasiveness of the fish due to its ability to survive subfreezing temperatures (Urquhart and Koetsier unpubl. data), hypoxia (Ip 2004) and seasonal habitat drying (Koetsier and Urquhart 2012).

Each of these issues poses potentially serious ecological threats to native aquatic fauna; yet the oriental weatherfish remains an overlooked and understudied part of many freshwater communities. Given the difficulty of eradicating any alien species once established (Vander Zanden et al. 2009, Horns 2010), the oriental weatherfish must be viewed as a permanent resident of the freshwater habitats where its introduction has

occurred. Consequently, the impacts of this cryptic fish on native faunal communities could be significant. Given the species' current world-wide distribution, its standing as a commercially available aquarium fish, and its display of traits characteristic of other highly successful invasive fishes, we predict that the oriental weatherfish will continue to invade new regions. Consequently, there exists a dire need for further research to decipher the ecological role of the oriental weatherfish within its invaded communities and the impacts its presence has on native (and endangered) biota.

Acknowledgements

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Table 3.1. Mass, frequency and numerical composition of stomach contents of 37 oriental weatherfish collected from water bodies connected with the Boise River, Idaho. Frequency and abundance indicated the fish were feeding primarily on benthic invertebrates and detritus.

Food Item	% by mass	Frequency	% by Number (SE)
Invertebrates	78.4		
Insecta			
Chironomidae		0.297	25.8 (13.9)
Hemiptera		0.027	2.0 (1.9)
Odonata		0.216	8.6 (3.1)
Formicidae		0.027	6.0 (6.1)
Disarticulated		0.405	13.2 (3.8)
Crustacaea			
Daphnia		0.027	0.7 (0.8)
Amphipoda		0.054	2.0 (01.6)
Annelida			
Oligochaetae		0.027	0.7 (0.7)
Mollusca			
Sphaeriidae		0.189	15.2 (7.9)
Gastropoda		0.135	7.9 (4.7)
Vegetation	14.6	0.243	12.6 (5.5)
Substrate	7.0	0.189	5.3 (2.3)
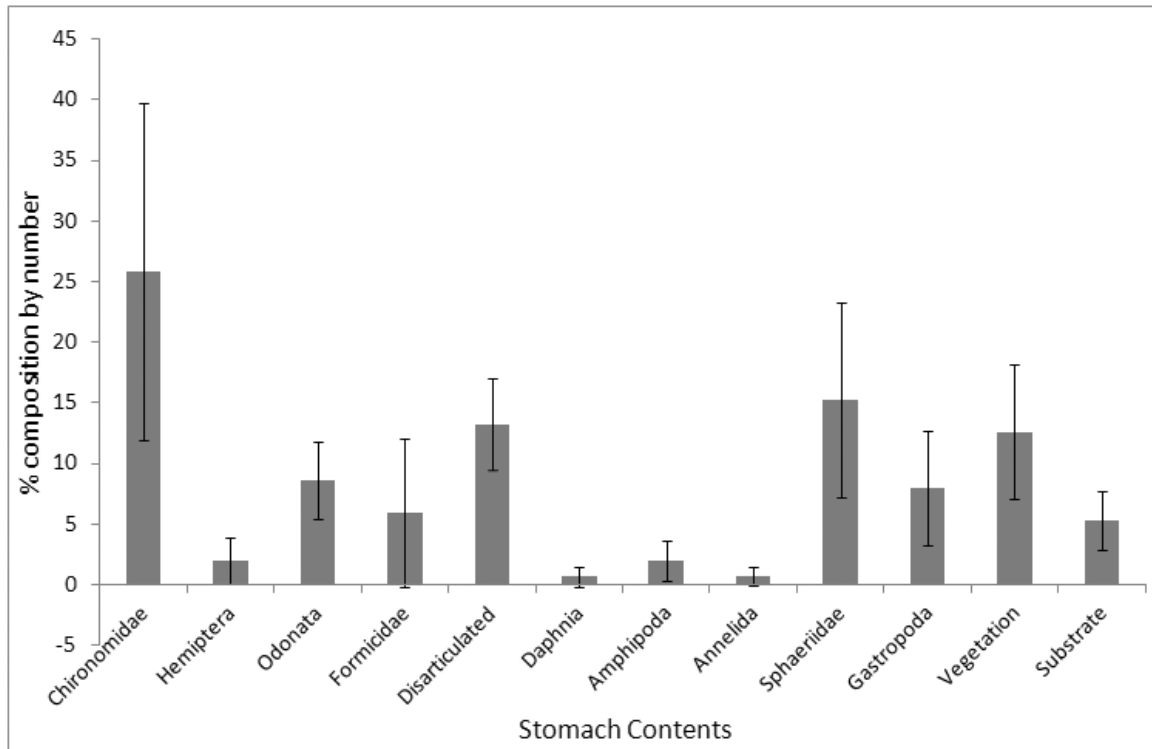
Figure 3.1. Mean percent composition by number (± SE) of stomach contents from 37 oriental weatherfish collected from water bodies connected with the Boise River, Idaho. Benthic invertebrates made up the majority of the food eaten, and chironomid larvae represent the most numerous prey item.
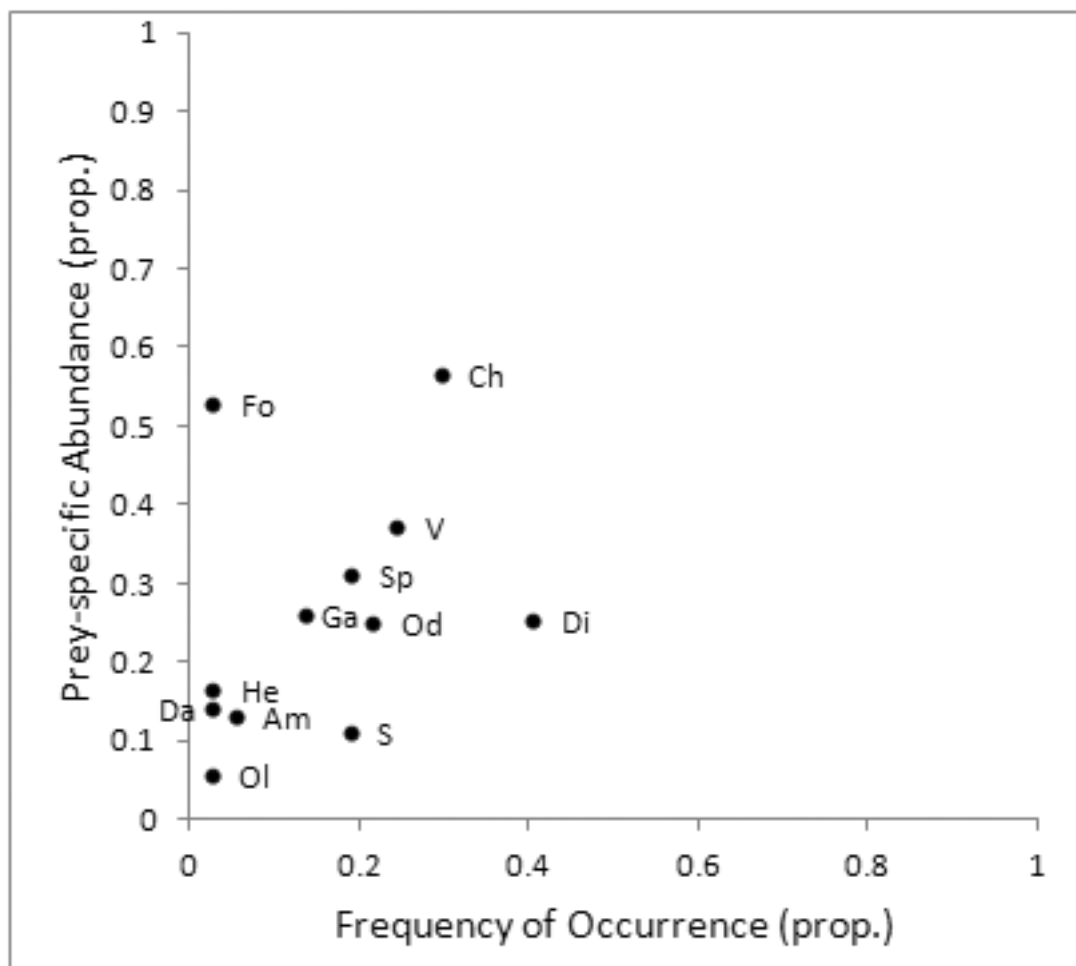
Figure 3.2. Abundance and importance of food items from stomach contents of 37 oriental weatherfish collected from water bodies connected with the Boise River, Idaho, USA. Grouping of food items along the diagonal indicates a generalized diet shared by the population (Amundsen et al. 1996). All food identities are labeled to the right of their associated datapoint, except *Daphnia*, which is to the left (Ch-chironomid larvae; He- hemiptera; Od- odonata; Fo- formicidae; Di- disarticulated arthropods; Da- *Daphnia*; Am- amphipoda; Ol- oligochaeta; Sp- sphaeriidae; Ga-gastropoda; V- vegetation; S- substrate).

CHAPTER FOUR: LOW TEMPERATURE TOLERANCE AND CRITICAL

THERMAL MINUMUM OF THE INVASIVE ORIENTAL WEATHERFISH

(*Misgurnus anguillicaudatus*) IN IDAHO, USA

Abstract

The oriental weatherfish (*Misgurnus anguillicaudatus*) is invasive to many countries around the world, but very little is known about the life history or environmental tolerances of this cryptic fish. As part of a larger life history study, we conducted an experiment designed to determine the Critical Thermal Minimum (CTmin) of oriental weatherfish collected from water bodies near Boise, Idaho, USA. Sixteen fish were placed into individual tanks in an environmental chamber in which air temperature was lowered over the course of 20 days until reaching 0°C. Air temperature was then held at 0°C for a range of 102 to 134 hours. This procedure was completed 3 times for a total of 48 fish. Water temperature in each tank decreased over the course of the experiment such that fish were exposed to temperatures ranging from 20°C to -3.64°C. Six of 48 fish died due to temperature related factors. Thirty eight of the remaining 42 fish survived exposure below the freezing point of fresh water and two fish survived full enclosure in ice with direct skin/ice contact. In absence of an observed LT_{50} we used logistic regression and observational data to extrapolate a CTmin of -1.8°C. The ability of oriental weatherfish to survive sub-freezing temperatures and being embedded in ice indicates physiological adaptation to cold that may include systemic antifreeze proteins.

Tolerance for low temperature and freezing resistance adds to a growing list of characteristics that make the oriental weatherfish a highly successful invasive species.

Introduction

Global biodiversity of aquatic ecosystems is threatened by invasive species (Olden et al. 2004; Villeger et al. 2011). In the United Stated alone, over 138 non-native fishes have been introduced (Pimentel et al. 2005). Many of these have established self-sustaining populations and are now viewed as a permanent part of the aquatic community (Taylor et al. 1984; Moyle and Light 1996). Establishment of invasive species often results in disruption of native communities and loss of native biota through habitat modification, competition, predation and pathogen introduction (Mills et al. 1994; Dextrase and Mandrake 2006; Vitule et al. 2009). Given the well-documented difficulties in eradicating an invasive species once established (Vander Zanden et al. 2009; Horns 2010), it is imperative to identify and characterize those species that have a history of becoming invasive, and that may also pose threats to native populations (Kolar and Lodge 2001; Simberloff 2003). The oriental weatherfish (*Misgurnus anguillicaudatus*) is one such invasive species.

The oriental weatherfish is native to southeastern Asia, extending from Myanmar to southern Russia, including the Japanese archipelago (Franch et al. 2008). Within its native range, oriental weatherfish are farmed as a food product, used as live bait and sold internationally by the aquarium pet trade. The species' standing as a popular ornamental fish may have aided its dispersal as an invasive species (Chang et al. 2009; Strecker et al. 2011). Currently, invasive populations are reported in at least eight countries, including

Philippines, Turkmenistan, Spain, and Italy (Freyhof and Korte 2005; Simon et al. 2006; Franch et al. 2008). Within North America, populations have been found in Mexico (Contreras and Escalante 1984) and at least 13 of the United States (Courtenay et al. 1987; Fuller et al. 1999; Tabor et al. 2001; Ashton and Ciccotto 2010). Australia banned importation of the oriental weatherfish in 1986, due in part to concerns regarding the fish's ability to colonize novel habitats and negatively impact native fish populations (Lintermans et al. 1990; Arthington and Bludhorn 1995), and dietary overlap with native species (Lintermans 2002). In Germany, an endangered native congener (*M. fossilis*) may be susceptible to habitat loss through competition with invasive populations of oriental weatherfish (Meyer and Hinrichs 2000; Freyhof and Korte 2005). Additional threats to native fish communities may include competition for invertebrate prey (Keller and Lake 2007) and direct predation of fish eggs and larvae (Logan et al. 1996). The oriental weatherfish is known to harbor 2 monogaean parasites and the LV-1 Birnavirus (Lintermans et al. 1990; Dove and Ernst 1998), a pathogen closely related to Infectious Pancreatic Necrosis Virus (Wolf 1988). Even with these potential threats, the oriental weatherfish remains an overlooked and understudied member of an increasing number of freshwater communities. Consequently, we know very little of this cryptic species' life history.

The oriental weatherfish is eurythermal, and has been reported living in water temperatures ranging from 2°C to 38°C (Axelrod and Schultz 1955; Lintermans and Burchmore 1996; Strecker et al. 2011). However, specific temperature ranges occupied by the oriental weatherfish vary between studies, and empirical data regarding direct temperature tolerance of the fish is lacking. Kubota (1961) suggested 20°C to 30°C as the

fish's preferred temperature range and Suzuki and Yamaguchi (1977) found that ideal temperature for egg development under aquaculture conditions was 25°C. Logan et al. (1996) attempted to determine the thermal tolerance of oriental weatherfish collected from Oregon, USA. All fish survived thermal challenge from 18°C to 6°C and from 6°C to 18°C, and no fish died when water temperatures were systematically lowered to 2°C. To our knowledge, no further empirical water temperature data has been published for the oriental weatherfish. While collecting fish for previous studies (Urquhart and Koetsier 2011; Koetsier and Urquhart 2012), we trapped individuals from water bodies in which water temperature ranged from 0°C to 38°C (Urquhart and Koetsier unpubl. data), and often broke through layers of ice to retrieve our traps during winter months. However, we do not know if the fish remained localized when water reached these temperatures. Trapped fish could have been moving from these extremes in search of more preferential conditions when caught.

Minimum water temperature is one abiotic habitat factor that has been used to predict the range limitations of other invasive fishes such as red–bellied piranha (*Pygocentrus nattereri*) in the United States (Bennett et al. 1997). Given the broad latitudinal range in which invasive populations of oriental weatherfish have been found (e.g., from Mexico to Michigan), we hypothesized that minimum water temperature may be an important abiotic factor that could function to limit further range expansion of the fish. Thus, the goal in this study was to identify the lowest temperature in which the oriental weatherfish can survive, and by extension answer the question: Is the invasion potential of the oriental weatherfish limited by minimum water temperature?

Traditionally, two methods have been employed to determine the low temperature tolerance of fish species (Wedemeyer et al. 1990). Incipient Lethal Temperature (ILT) involves acclimatizing fish to a fixed temperature, then plunging them into water several degrees colder and evaluating their viability. Critical Thermal Minima (CTmin) involves acclimatizing fish to a fixed temperature, then decreasing water temperature at a constant rate until the fish lose the ability to control their movements. ILT and CTmin are calculated as the temperature at which 50% of the fish exposed to that temperature lose the ability to escape and will ultimately die (LT_{50}). However, neither method accurately depicts natural temperature regimes experienced by a fish (Bennett and Judd 1992); rarely does temperature of natural water bodies suddenly decrease tens of degrees, as faced in ILT trials (but see Bennett and Beitinger 1997, and Marti-Cardona et al. 2008). Even the slower decrease of 1°C per hour, as often used in CTmin tests, may be unrealistic for natural environments. Both ILT and CTmin temperatures are highly dependent upon the fishes' acclimation temperatures (Beitinger et al. 2000), such that lower acclimation temperature often results in a lower calculated LT_{50}.

With these limitations in mind, we applied a temporally extended CTmin approach to define the lowest temperature survival limit of a sample of oriental weatherfish collected from an invasive population in and around Boise, Idaho, USA. By extending exposure time at each temperature, we hoped to better mimic natural conditions to which oriental weatherfish may be exposed within their invaded habitats.

Methods

Between April- 2008 and July- 2009, we collected 586 oriental weatherfish from irrigation canals and ponds, each with a seasonal connection to the Boise River, in southwestern Idaho. Fish were collected using vinyl coated, steel mesh minnow traps, outfitted with thermograph data loggers (HOBO®; Onset Computer Corporation). Traps were approximately cylindrical and measured 42 cm x 22 cm with a 2.5 cm opening at the apex of an inverted cone in each end and were baited with approximately 30g dry commercial dog food. Water temperature was recorded by the data loggers hourly throughout the entire collection period. During this time, water bodies were exposed to seasonal temperature variation and, during winter months, were often covered in ice up to several cm thick.

Fish were removed from traps weekly, and transported alive to the Aquatic Ecology Laboratory at Boise State University where they were sexed (Urquhart and Koetsier 2011) measured for total length (TL), and wet weighed to the neared 0.00 g. Approximately half of all fish collected from all locations throughout the 16 month sampling period were placed in two communal holding tanks in a climate-controlled greenhouse. Communal tanks were constructed of clear acrylic (43 x 73 x 37.5 cm) with metal-mesh screened tops, and fitted with pour-over aquarium filters and air stones. Approximately 3 g commercial pellet fish food was added to each tank by automatic feeders twice daily and fish fed ad libitum. Tanks were cleaned of excess algae and water changed weekly. Fish were housed in these conditions until utilized in experiments and surviving fish were returned to a "used fish" communal tank and not utilized for any subsequent experimentation. Air temperature in the greenhouse was held at 20°C, and

fish were exposed to ambient water temperature (approximately 18°C - 22°C) and natural lighting regime for at least 1 month prior to being utilized in this study.

Our experimental design consisted of 16 clear acrylic tanks (18 x 28 x 17 cm) placed on four shelves (four tanks per shelf) within an environmental chamber (internal dimensions 71 x 135 x 56 cm). The chamber was outfitted with full spectrum lighting and a two-channel microprocessor unit programmed to control temperature and photoperiod (Figure 4.1). Each tank was partially filled with 3.0 L artificial pond water (0.03 g/L $NaHCO_3$, 0.35 g/L NaCl, 0.007 g/L CaCl in dH_2O; pH 8.0, conductivity 800 µS/cm) and was outfitted with a single air stone and thermocouple connected to an electronic data logger (21x micrologger, AM416 multiplexer; Campbell Scientific). Photoperiod was set at 12 hours light/dark and air temperature inside the chamber and water temperature of each experimental tank were recorded hourly, for the duration of each experimental trial. We completed three sequential trials such that the first began on October 9, 2009 and the third trial was completed on January 2, 2010.

At the start of each trial, we used a random number generator or coin-flip to identify which of the two communal tanks from which to select a fish. We hand-netted a single fish and used the same method to determine whether to use or release that fish. In this manner, we randomly selected 16 fish and each was added to one of 16 experimental tanks within the environmental chamber. Air temperature within the environmental chamber was set at 20°C and held there until the addition of fish at the start of each trial. Fish were not fed and water was not changed in the experimental tanks during the duration of each trial.

At the beginning of each experimental trial, air temperature was held at 20°C for the first 24 hours then decreased by 2°C, over one hour. Air temperature was then held constant for the next 23 hours. Following this regimen, air temperature inside the chamber was lowered 2°C every 24 hours until reaching 6°C; after which, air temperature was decreased 1°C every 24 hours until reaching 0°C on day 14. Air temperature was held at 0°C for 102 hours in trial one, and 110 hours in trial two, and then increased to 10°C for 24 - 48 hours to thaw any ice that had formed within the tanks. Due to low mortality rates in our first and second trials, we increased the 0°C hold time to 134 hours in our third trial.

Fish were assessed daily for viability by visual observation of opercular movement or gently probing each fish with a stainless steel rod to elicit a swimming response. In the cases where ice prohibited probing, we tapped on or gently shook the tank and observed any responsive movement. Dead fish were removed daily (if not inaccessible due to ice) to prevent decay. Each dead fish was visually inspected for condition in case external signs denoting probable cause of mortality (other than temperature exposure) could be determined, such as compounding fungal infections or parasites. At the termination of the final warming phase of the temperature treatment, surviving fish were removed from the experimental tanks and held at 20°C in a communal tank for a minimum of 72 hours. After this period, it appeared that no further mortality would result from thermal stress inflicted during the experiment and fish were moved to a "used fish" tank.

During the experiment, four fish showed signs of a fungal infection consistent with Saprolegniasis (D. Burton, Idaho Department of Fish and Game, personal

communication). Two of these fish died during the experiment and were excluded from the analyses because the fungus became a compounding factor. The other two fish did not die and signs of the fungal infection abated in these fish during the post-experiment 20°C observation period. These two fish were included in subsequent analyses because regardless of additional stress due to fungal infection, temperature exposure did not kill these fish.

We compared lowest mean water temperature of experimental tanks between trials using Analysis of Variance (ANOVA). We also compared fish mortality between experimental trials using logistic regression. To determine whether it was low water temperature experienced by the fish or cumulative time spent below freezing temperature that caused fish mortality, we modeled minimum water temperature, cumulative time below 0°C and an interaction of these against fish mortality in a logistic regression. We used corrected Akaike's Information Criterion (AICc) values to select what combination of these variables best predicted fish mortality during our experiment.

We did not reach a lethal temperature at which 50% of the fish died (LT_{50}) at the lowest water temperatures recorded during this experiment. In absence of these data, we used logistic regression analysis to model the probability of death occurring at each temperature to which the fish were exposed (Berkson 1951; Tsutakawa 1982). We used the resulting linear equation to calculate the temperature at which log-odds (logit) of dying was equal to log-odds of survival. This temperature became our predicted LT_{50} and we further calculated the 95% confidence interval to determine the range in which the true CTmin for this group of fish resides.

Results

Eight of 48 fish died during the course of our experiment, two of which were excluded from analyses due to confounding fungal infection. Additionally, one fish appeared to have lost the barbels around its mouth during low temperature exposure.

Minimum water temperature differed between trials ($F_{2,43} = 6.97$, $p < 0.05$) such that mean minimum temperature of tanks in trial three (-1.30°C, SE = 0.47°C) was lower than that in trials one (-0.50°C, SE = 0.31°C) and two (-0.45°C, SE = 0.24°C) (Figure 4.2). This difference was attributed to the longer hold time at 0°C air temperature during the third trial, as the extended hold time allowed individual tank water to reach lower temperatures. Fish mortality differed between trial as well ($X^2 = 8.35$, $p < 0.05$); five of the six fish included in analyses died in trial three compared to a single death in trial one and no deaths in trial two. As a result of these trial effects, experimental trials were not treated as replicates, and instead all tanks in all trials were pooled for analyses. Model selection, using AICc values (Table 4.1) showed that minimum temperature and cumulative time below 0°C both affected fish mortality such that probability of dying increased with decrease in temperature and an increase of time exposure below 0°C. However, these variables are also highly correlated (Pearson R = -0.74) such that water reached lower temperatures as time below 0°C increased.

All fish in this experiment were exposed to water temperatures below 0.45°C and all but four tanks reached water temperatures below 0.00°C. Minimum temperature for surviving fish (mean = -0.54°C, SE = 0.08°C) differed significantly from that of fish that died (mean = -2.12°C, SE = 0.37°C) ($X^2 = 5.90$, $p < 0.05$). Time exposure at or below 0°C for surviving fish ranged from one to 145 hours (mean = 62.4 hrs, SE = 6.3 hrs) and

for dead fish ranged from 100 to 183 hours (mean = 149.8 hrs, SE = 13.8 hrs) and also differed significantly (X^2 = 5.06, p < 0.05). Thirty five fish survived water temperatures between 0.00°C and -1.00°C and five of these fish survived these temperatures for longer than 100 hours (Figure 4.3). Only one fish exposed to this temperature range died. Six of seven fish exposed to temperatures between -1.00°C and -2.00°C also survived.

Logistic regression of minimum temperature versus fish mortality provided a relationship as defined by the equation $y = -3.604x - 6.331$, where y represents the log-odds ratio (logit) of death to survival, and x represents minimum water temperature experienced by a fish. Where $y = 0$, log-odds of death is equal to log-odds of survival; therefore, this point also represents the point where 50% of the fish should die (LT_{50}). Solving for x, at this point ($y = 0$), we calculated the CTmin of fish in this experiment as -1.76°C (SE = 1.05°C), with a 95% confidence interval of -3.81°C to 0.30°C.

Most of the experimental tanks froze along the top and tank margins such that fish were enclosed within a small liquid water refuge surrounded by ice on all sides. All fish that were enclosed in ice, but with liquid water between themselves and the ice, survived with no apparent ill effects of being exposed to such conditions. Several fish survived direct contact with ice as they became partially trapped with water surrounding other parts of their bodies (Figure 4.4). One fish was encased in ice by the head but with the rest of its body surrounded by liquid water for at least 24 hours and survived. Seven fish became wholly encased and in direct contact with ice for various amounts of time ranging from 34 hours to 109 hours. Two of these fish survived encasement for 34 hours and 54 hours, respectively. The other five fish died while encased for 78 hours or more.

Discussion

Oriental weatherfish in our experiment survived extended exposure to temperatures below 0°C and several fish survived partial or full entrapment and direct contact with ice. Five fish showed signs of damage potentially caused by thermal stress (fungal infections and loss of barbels). Three of these fish survived, and we could not unequivocally attribute these conditions to temperature exposure. However, thermal stress can lower immune response and contribute to pathogen infections (Bly et al. 1997; Engelsma et al. 2003; Goodwin et al. 2009; Ibarz et al. 2010). Thus, continued exposure to the low temperatures reached in this experiment may increase the rate of fish mortality. Those fish that died during our experiment were exposed to water temperatures between -0.94°C and -3.64°C and most of them (five of six fish) died while fully encased in ice for 78 hours or more.

While we were unable to reach a directly observed LT_{50} in this experiment, our model indicates that the true lethal temperature is at or below 0°C. Because of high variation in minimum temperatures and the paucity of fish mortality during our experiment, the 95% confidence interval of our calculated CTmin spans a large temperature range (-3.81°C to 0.30°C). However, no fish exposed to temperatures lower than -1.99°C survived, and no fish died in temperatures above -0.94°C. Coupling these apparent limitations with our model-extrapolated CTmin (-1.76°C), it is reasonable to assess a range of water temperature between -1°C and -2°C as the approximate lowest limit for survival of oriental weatherfish from our sampled population.

The freezing point of intracellular fluid in most fishes is approximately -0.7°C (Helfman et al. 2009). However, many fish have adapted physiological mechanisms to

survive subfreezing temperatures. Colligative properties of solutes in blood plasma and intracellular fluid lower the freezing point of body fluids in many fishes (DeVries and Cheng 2005). However, fishes that utilize colligative properties to survive subfreezing temperatures remain in a supercooled state and are often at risk of direct ice contact (Scholander et al. 1957; Scholander 1971). Such contact catalyzes the formation of ice crystals, causing virtually instantaneous freezing of tissues. By contrast, some fishes have evolved production of antifreeze proteins (AFP) that non-colligatively prevent ice formation within body fluids, allowing the fish to survive even direct skin contact with ice (Fletcher et al. 2001; DeVries and Cheng 2005). Fish, eggs and larvae with high AFP concentrations can withstand direct ice contact with little or no detrimental effects (Valerio et al. 1992). Additionally, AFPs can be induced by environmental conditions and concentrations vary geographically and by season, depending on the fishes' needs (Goddard et al. 1992, 1999; Raymond and Hassel 2000).

Published reports of AFP presence in fish have predominantly focused on Arctic and Antarctic marine species. However, Yamashita et al. (2003) found AFPs in freshwater Japanese smelt (*Hypomesus nipponensis*) that were similar to those found in marine fishes. These findings indicate that freshwater fish may also produce these proteins when needed. Several fish in our experiment came into direct contact with ice and survived. One fish survived with its head encased in ice for at least 24 hours, and two fish survived whole body encasement for 34 and 54 hours. Each of these fish fully recovered when thawed and showed no indication of damage. Although determining systemic presence of AFPs was beyond the scope of this study, it is clear that some

adaptive mechanism is at work in oriental weatherfish, allowing survival of sub-freezing temperatures and direct skin/ice contact.

Because the freezing point of freshwater is 0°C, and it is densest at 4°C, most freshwater fishes never encounter temperatures low enough to threaten freezing of body fluids. However, oriental weatherfish reportedly burrow into the substrate during seasonal habitat drying (Kubota 1961; Koster et al. 2002; Tsui et al. 2002; Ip et al. 2004) and can survive in dry soil for several months (Koetsier and Urquhart 2012). Many of our sample fish were collected from irrigation canals that are seasonally dewatered and remain dry throughout the winter months. While collecting fish for this study, we were told of anecdotal accounts from farmers who claimed finding oriental weatherfish alive and active while removing soil from dried irrigation ditches during the winter. If oriental weatherfish are utilizing their burrowing capabilities to remain localized during winter drying, survival of periodic subfreezing soil temperatures may be a necessary part of their overwintering strategy.

In southwestern Idaho, subfreezing air temperatures are common during the winter. Ground temperatures may reach sub-freezing up to 8 cm below the surface, but deeper soil temperatures seldom reach below 0°C (National Resource Conservation Service 2012). Kubota (1961) found that most oriental weatherfish burrow less than 10 cm into the substrate as an overwintering strategy when rice paddies and streams dry. However, within the native region studied, year-round soil temperatures exceeded 5°C, never approximating freezing temperature. If this characteristic depth and behavior is retained within invaded regions, then subfreezing temperatures may come into effect as a possible cause of winter mortality. By contrast, if oriental weatherfish burrow to a depth

below that of frozen substrate, they may avoid periodic ice formation. However, to our knowledge no published study of burrowing depth exists outside of the fish's native range. In habitats where substrate temperatures reach freezing temperatures below 10 cm depth, oriental weatherfish may survive ice exposure, but the compounding effect of thermal and desiccation stresses may limit survivorship. Overwintering success may therefore be limited to permanent water bodies that function as strongholds, from which ephemeral and temporary water bodies may be recolonized. In habitats that are not dried or do not completely freeze during winter, oriental weatherfish survival may resemble that of other freshwater fishes; simply remaining at or near the bottom of the water body where temperatures seldom approach freezing, regardless of air temperatures (DeVries 1971; Helfman et al. 2009).

Successful invasive fish species have been characterized as possessing certain life history traits. Among these are long life, early maturation, high fecundity, and broad thermal tolerance (Townsend 1996; Marchetti et al. 2004; Vila-Gispert et al. 2005). Each of these characteristics functions to advance the survival and establishment of individuals introduced into a foreign habitat. The oriental weatherfish possesses many of these traits. The fish has a life span of up to seven years in the wild (Urquhart and Koetsier unpubl. data), and Suzuki (1983) experimented with captive fish up to 10 years old. The fish displays rapid growth to maturity and is sexually mature within one year of age (Kubota 1961; Lei and Wang 1990). At which time the fish is capable of very high reproductive output, and may spawn multiple times, over a protracted mating season (Suzuki 1983; Urquhart and Koetsier unpubl. data).

Oriental weatherfish use a range of behavioral and physiological characteristics to survive hypoxic water conditions and aerial exposure including surface air breathing and cuticular volatilization of nitrogenous wastes (Chew et al. 2001; Tsui et al. 2002; Ip et al. 2004; McNeil and Closs 2007). The fish can survive months of habitat drying, and resist desiccation by burrowing into the substrate; waiting out these conditions in a small excavated chamber, several cm below the ground surface (Koetsier and Urquhart 2012). Adding to this list, our present study indicates the ability of oriental weatherfish to survive sub-freezing water (and perhaps soil) temperatures.

Ecological impacts of invasive populations of the oriental weatherfish are as yet uncertain. However, given the species' current world-wide distribution, its standing as a commercially available aquarium fish, and its display of traits characteristic of other successful invasive fishes, we predict that the oriental weatherfish will continue to invade new regions. Additionally, our data suggests that minimum water temperature may not function to limit further range expansion of the fish. Consequently, we suggest that future studies focus on the specific ecological impacts of the oriental weatherfish on native fish and invertebrate communities within habitats where this invasive fish has become established.

Professor Emeritus of Boise State University for introducing us to this novel fish species.

All field and laboratory procedures were carried out in accordance with the American

Fisheries Society publication, Guildelines for the Use of Fishes in Research (2004).

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Table 4.1. Model selection of parameters affecting fish mortality of oriental weatherfish exposed to minimum water temperatures. AICc values indicated that CTmin and Time at or below 0°C both affected fish mortality such that the probability of dying increased as water temperature decreased, and time of exposure to water temperature below freezing increased.

| Model	AICc	ΔAICc	K	Weight	LL
Minimum, Time ≤ 0°C	18.88	0.00	4	0.5777	-4.95
Minimum, Time ≤ 0°C, Minimum * Time ≤ 0°C	21.34	2.46	5	0.1685	-4.92
Minimum	21.53	2.66	3	0.1531	-7.48
Time ≤ 0°C	22.37	3.49	3	0.1007	-7.90
null	39.90	21.02	2	0.0000	-17.81
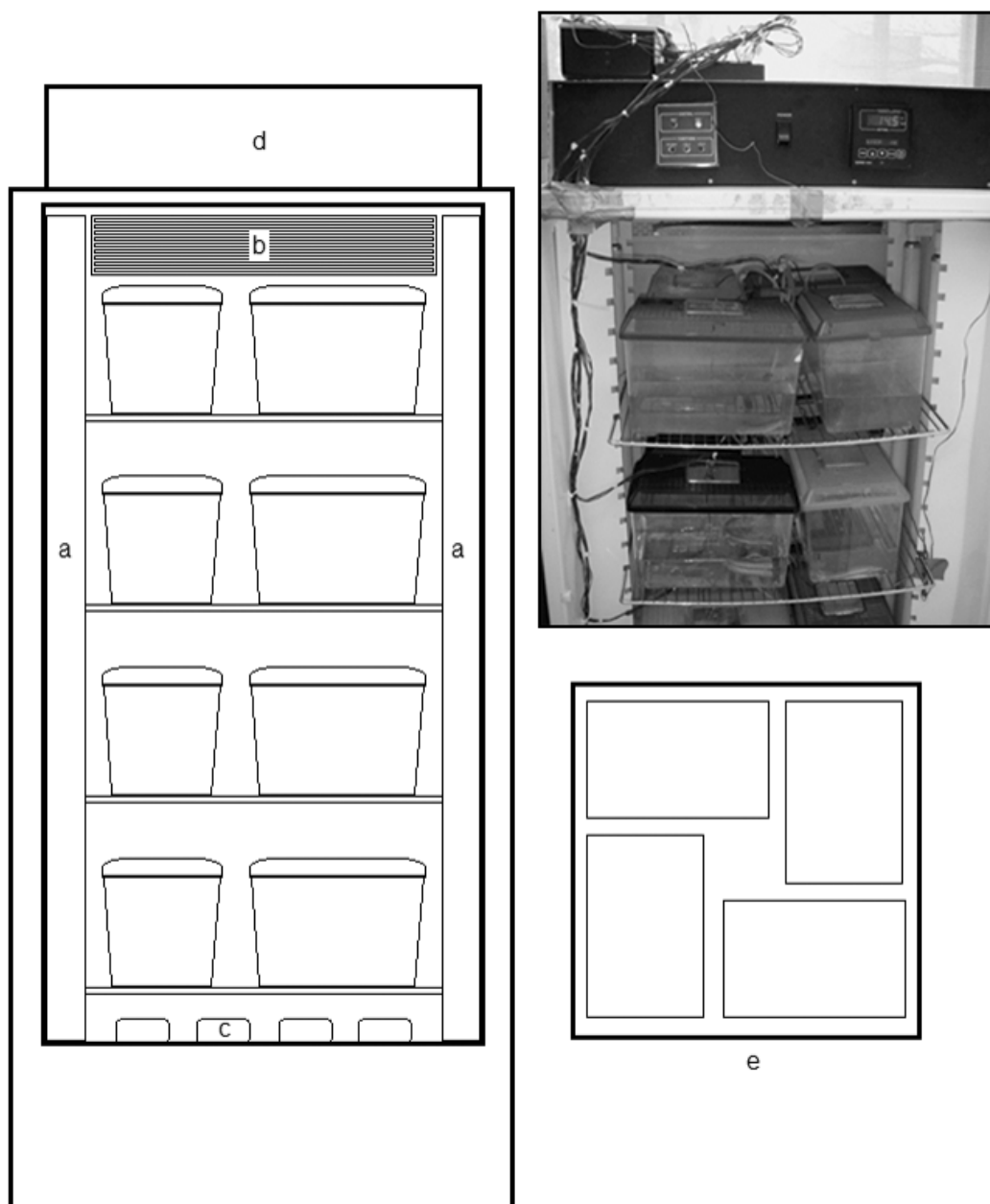
Figure 4.1. Experimental setup for determining the CTmin of oriental weatherfish taken from water bodies near Boise, Idaho, U. S. A. An environmental chamber was outfitted with 4 shelves, each holding 4 acrylic fish tanks. Each tank contained one randomly selected fish, 3L artificial pond water, a single airstone, and thermocouple temperature probe. Photoperiod was controlled at 12 hr light/dark, and air temperature was lowered inside the chamber over 14 days then held at 0°C for 102 – 134 hrs. a) full spectrum fluorescent lights; b) excurrent cold air vent; c) aquarium air pumps; d) chamber control unit; e) tank arrangements on each of 4 shelves. Inset photo shows inside of the chamber with tanks, air hoses and wiring of thermocouples to the datalogger and multiplexer on top of the chamber.
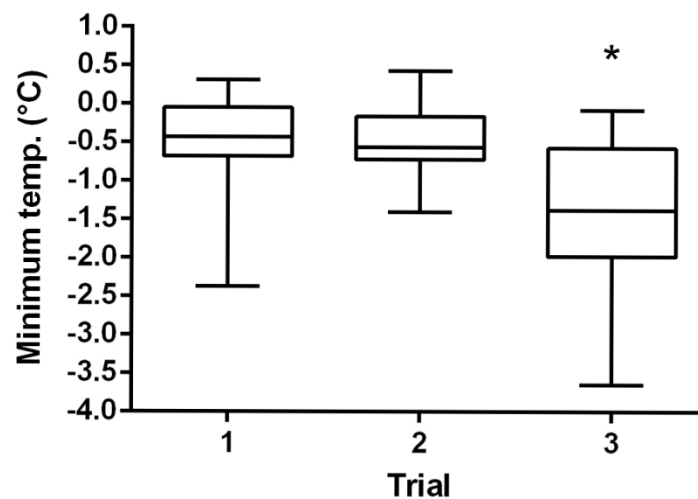
Figure 4.2. Median and quartile plots of minimum water temperatures in each of three experimental trials. Whiskers display maximum and minimum values. Experimental tanks in Trial 3 reached significantly lower water temperature ($F_{2,43} = 6.97$, $P = 0.002$) than tanks in Trial 1 or Trial 2, therefore, trials were not treated as replicates. * indicates a statistically significant difference.
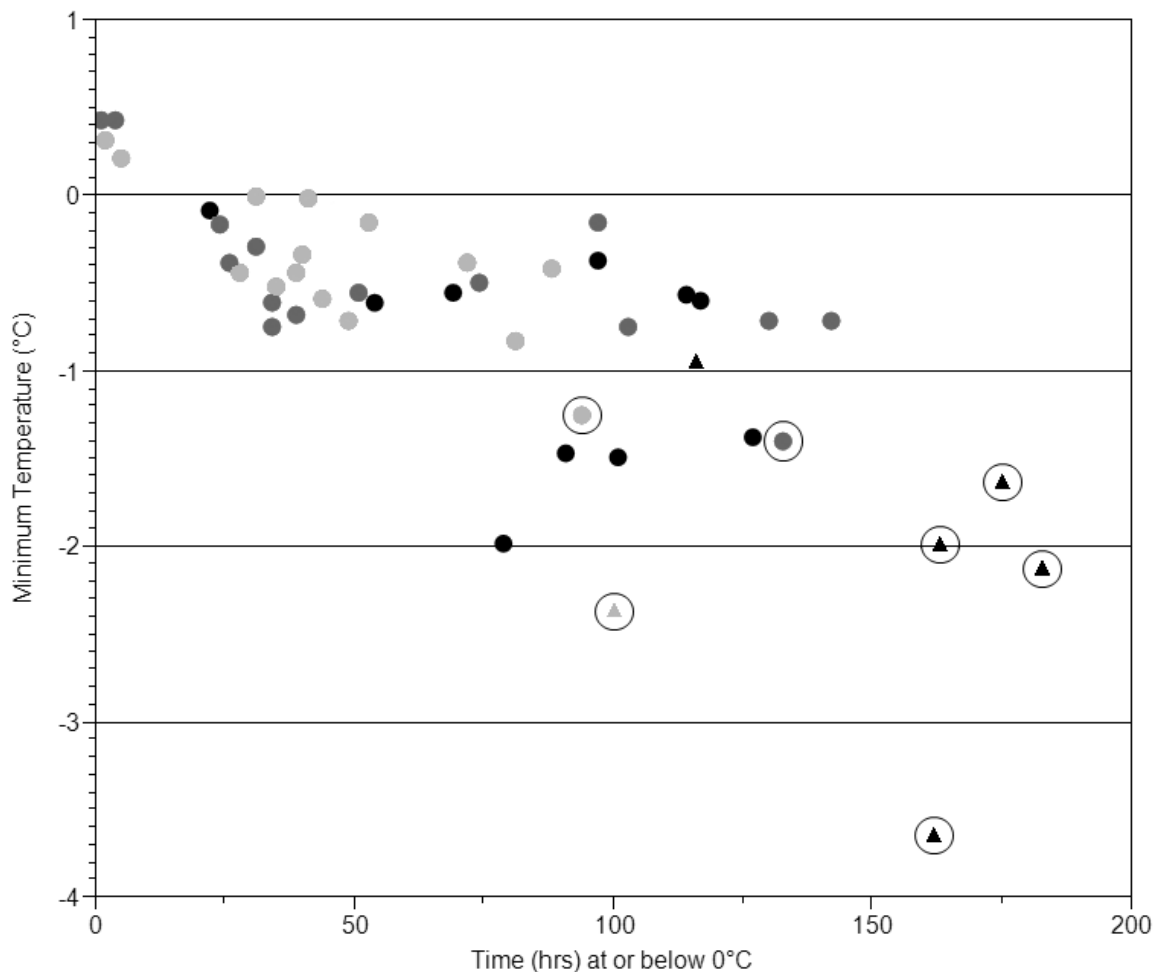
Figure 4.3. Mortality of oriental weatherfish during exposure to low water temperatures. Fish that died during the experiment were exposed to significantly lower temperatures (mean = -2.12°C, SE = 0.37°C) than those that survived (mean = -0.54°C, SE = 0.08°C) (X2 = 5.90, P < 0.05). Fish that died were also exposed to subfreezing temperatures for longer time (mean = 149.8 hrs, SE = 13.8 hrs) than those that survived (mean = 62.4 hrs, SE = 6.3 hrs) (X2 = 5.06, P < 0.05). Greyscale markers represent fish from different experimental trials; light grey- Trial 1, dark grey- Trial 2; black- Trial 3. Round markers represent surviving fish. Triangle markers represent dead fish. Circled markers represent fish which were wholly encased in ice during the experiment.
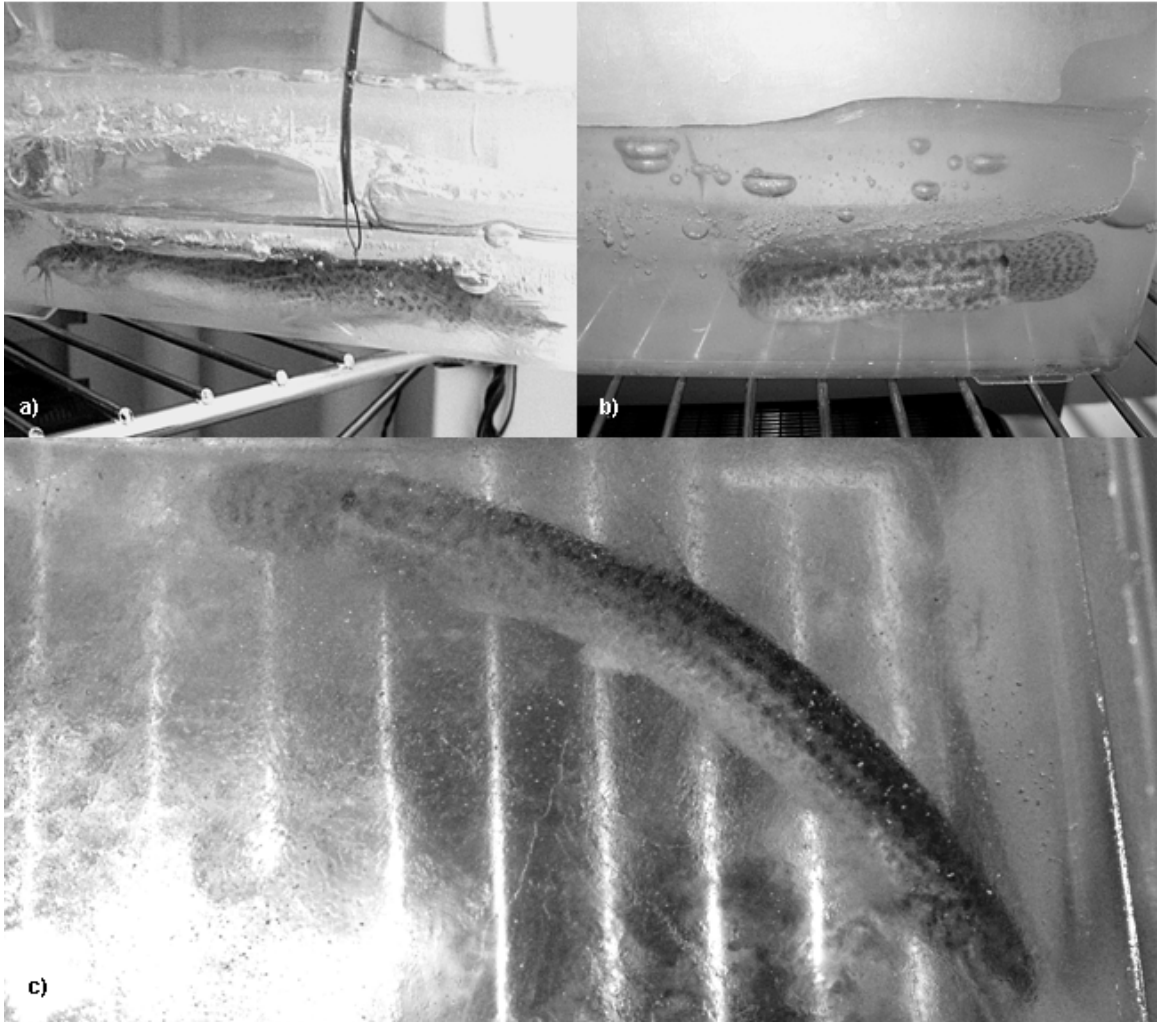
Figure 4.4. Oriental weatherfish survived direct contact with ice during the course of the experiment. a) Several fish became enclosed within a liquid water refuge surrounded by ice. b) This fish became partially entrapped in ice by its head with liquid water surrounding the body and tail. c) This fish was wholly encased in ice for 34 hours and survived when thawed. All fish in these pictures survived with no apparent ill effects of thermal minima or direct skin contact with ice.

CHAPTER FIVE: CONCLUSIONS

Invasive species have been identified as a serious threat to world-wide biodiversity (Lockwood et al. 2000; Olden et al. 2004; Keith et al. 2009; Villeger et al. 2011). The economic cost associated with establishment and spread of invasive populations is estimated at billions of dollars annually (Pimentel et al. 2005). Around the world, invasive species are continuously being introduced (both intentionally and through accidental escapes) into novel habitats with varied and often unpredictable effects (Moyle and Light 1996a, Simberloff and Stiling 1996). Most species introductions fail, even when organisms are intentionally released (Mack et al. 2000, Zenni and Nuñez 2013). However, some organisms survive and persist, eventually creating self-sustaining populations that increase in number and spread into neighboring habitats (Sakai et al. 2001). Considerable effort has been expended to define the biotic and abiotic factors that lead to successful establishment and spread of invasive species, and this is no less true within freshwater aquatic ecosystems.

While highly predacious and costly aquatic invaders such as the northern snakehead (*Channa argus*), red-bellied piranha (*Pygocentrus nattereri*) and zebra mussels (*Dreissena polymorpha*) catch the public eye, other lesser known invaders succeed in establishing new populations, secretively spreading within invaded ecosystems. The most successful of these aquatic invaders share common life history traits that aid in this process. Some of these characteristics are a long lifespan, multiple

spawning events with high reproductive output, broad environmental tolerance, and a generalized, omnivorous diet (Moyle and Light 1996a, Marchetti et al. 2004). The oriental weatherfish is one such invasive fish that embodies many of these characteristics.

An introduced species with a long lifespan has a greater chance of becoming established than one that dies shortly after introduction (Vila-Gispert et al. 2004). In the same fashion, a species that reproduces many times, over a protracted mating season has a higher probability of becoming invasive than one with limited reproductive events and few offspring (Ruesink 2005). The oriental weatherfish is a long-lived fish with a life span of up to 7 years in the wild. Young fish grow rapidly and reach maturity within one year of hatching. Upon reaching maturity, each female fish may participate in multiple spawning events over a protracted spawning season. Throughout this summer long mating period, females may release upwards of 40,000 eggs. Fish remain fecund throughout their lifetimes and this high reproductive output may aid in the establishment and rapid spread of invasive populations.

The ability to adapt to a wide range of abiotic environmental variables (e.g., temperature, fluctuations in water availability) improves survival of an introduced fish within diverse habitats, thus increasing the probability of establishment and invasion (Moyle and light 1996b, Sakai et al. 2001, Kolar and Lodge 2002). Invasive fish that are adapted to a broad, heterogeneous native range are often more successful than those with a narrow, homogenous native range (Marchetti et al. 2004). The native range of the oriental weatherfish spans from tropical regions (Vietnam and Myanmar) to as far north as southern Russia. This broad latitudinal range undoubtedly exposes the fish to a diverse set of habitat variables throughout its native range. The fish's ability to adapt to these

various conditions appears to be retained within invasive populations. The oriental

weatherfish can survive subfreezing water temperatures and direct contact with ice. This

ability may allow survival of the fish while overwintering in seasonally dried water

bodies. Additionally, the oriental weatherfish is a surface air breather (McMahon and

Burggren 1987), and can burrow into the substrate and survive desiccation of the

surrounding soils for months at a time (Koetsier and Urquhart 2012).

Within invaded habitats, food availability may differ from that within a fish's

native range. The ability to adapt and switch food sources is a characteristic shown in

highly successful invaders such as the threespine stickleback (*Gasterosteus aculeatus*;

Adachi et al. 2012), pike killifish (*Belonesox belizanus*; Harms and Turingan 2012) and

the round goby (*Neogobius melanostomus*; Kornis et al 2012). Eating a generalized or

omnivorous diet is also a beneficial attribute that aids in survival and establishment of

new populations (Moyle and Light 1996a, Ruesink 2005). Within its invaded range, the

oriental weatherfish eats a generalized diet consisting primarily of aquatic invertebrates

and detritus. The ability to find and consume food using tactile sense and chemoreception

(Watanabe and Hidaka 1983) aid the fish in utilizing whatever dietary components are

available. This omnivorous and non-specialized diet may be another key to the success of

the fish within its invasive range.

Along with characteristics that are common to other highly successful invaders,

the oriental weatherfish presents several potential ecological threats to native fauna. The

oriental weatherfish has been shown to decrease invertebrate communities that may be a

necessary part of the diet of native fishes and other vertebrates (Keller and Lake 2007).

The oriental weatherfish is the known vector of introduction of at least one monogaean

parasite (Dove and Ernst 1998) and is a carrier of a viral pathogen (LV-1 Birnavirus) that may be detrimental to native fishes. At least one endangered fish (*Misgurnus fossilis*) within the oriental weatherfish's invaded ecosystems shares a common life history, habitat preference and diet (Meyer and Hinrichs 2000). This native congener may lose crucial habitat to its invasive sister species if the current pattern of range expansion continues. Throughout the world, new populations of oriental weatherfish continue to be reported (e.g., Abilhoa et al. 2013, van Kessel et al. 2013). Given the ongoing distribution of the oriental weatherfish as an aquarium pet, and the propensity for release of unwanted pet fish (Strecker et al. 2011), new populations will undoubtedly continue to become established.

Currently, invasive populations of oriental fish have been reported in at least ten countries, on six continents, including parts of Oceana. The life history traits displayed by the oriental weatherfish within these areas are common to other highly successful invaders. The oriental weatherfish is a long-lived invader that reaches maturity early in life, is highly fecund and reproduces multiple times annually, over a prolonged mating season. It feeds on a generalized diet and can sustain itself within inhospitable environmental conditions that may limit the survival of native or other invasive fish. It poses several potential ecological threats to native faunal communities, yet the fish remains highly understudied in ecosystems where it is invasive. It is clear that the oriental weatherfish possesses the hallmark characteristics of a highly successful invasive fish. What remains to be learned is how native faunal communities are being affected by this cryptic but widespread invader.

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