

Ecology and conservation of *Garra ghorensis*, an endangered freshwater fish in Jordan

Nashat A. Hamidan

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Abstract

The focal species of the research was the freshwater cyprinid fish *Garra ghorensis*. Endemic to the southern Dead Sea basin of the Middle East, it is 'Red listed' by the IUCN as 'endangered'. It inhabits the small rivers of the basin ('wadis'), existing within fish communities of very low species diversity. The aim of the research was to inform conservation strategies for the species through investigations into their phylogeny, current distribution, life history traits and feeding relationships.

Analysis of the mitochondrial DNA of *G. ghorensis* with other fishes of the *Garra* genus tested two contrasting hypotheses on their biogeographic origin: whether they were descended from *Garra tibancia* in the Arabian Peninsula or from a common ancestor shared with *Garra rufa*, which would have indicated dispersal from the Mediterranean basin and Mesopotamia. The phylogenetic tree clearly indicated that *G. ghorensis* shared a common ancestor with *G. rufa* and thus was of Mediterranean origin. These phylogenetic analyses were then important for interpreting *G. ghorensis* biogeography in relation to their natural range and current distribution. Surveys completed in 2010 provided data on their spatial distribution; this distribution was at least partially explained by historical geological and water salinity changes of the proto-lakes of Lake Samra and Lisan. These surveys also revealed that during the 2000s, there had been little change in *G. ghorensis* distribution, with populations still present in wadis that were recorded in 2002. However, at the surveyed sites, some alterations to the physical habitats and hydrology of the wadis were apparent, such as construction of impoundments.

To assess the life history traits and feeding relationships of *G. ghorensis*, three locally abundant populations were studied. These were an allopatric population, a population sympatric with the native cyprinid *Capoeta damascina* and a population sympatric with

the invasive cichlid *Oreochromis aureus*. The allopatric and sympatric native populations were present in wadis with minimal habitat disturbance, whereas the sympatric invasive population was present in a wadi with substantial alteration, including some impoundments that deepened the main channel and reduced the flow. Analyses of ages, growth rates and reproductive traits revealed that life spans, growth rates and reproductive investment were greatest at this disturbed site, despite being relatively altered from the apparently preferred habitat of the species. These results suggested that providing the hydrological disturbance at sites where *G. ghorensis* is present still enables the completion of their life cycle then their populations can withstand some aspects of habitat disturbances from anthropogenic activities.

The feeding relationships of *G. ghorensis* were then assessed in relation to the presence of *C. damascina* and *O. aureus* in two of the sites, and used a combination of stomach contents analyses and stable isotope analysis. Results from both methods revealed whilst there were some overlaps in the trophic niches of the fishes, diets were based mainly on detritus and algae. These items are rarely limiting in freshwaters and thus whilst resources were shared, it was unlikely to result in high levels of inter-specific competition.

Thus, an important ecological feature of *G. ghorensis* populations is their plasticity in life history traits and their resource use that enables some adaptation to disturbed environments. This suggests that their conservation management does not necessarily have to return their habitats to pristine conditions, as their adaptive capacity should enable some adaptation to the new conditions and thus continued population sustainability. Consequently, providing that development schemes prevent the destruction of the key habitats required for the completion of the *G. ghorensis* lifecycle, then their populations could remain sustainable in the face of continued development in the region.

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Dedication

This thesis is fully dedicated to the soul of my mother who drew her last breath during this work. I always inspired by her insistence, seriousness, and patience.

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Author's declaration

I confirm that this thesis is all my own work, with the following exceptions:

Chapter 3 was published and was written in collaboration with Geiger, M, and Freyhof, J. as:

Hamidan, N., Geiger, M., and Freyhof, J., 2014. *Garra jordanica*, a new species from the Dead Sea basin with remarks on the relationship of *G. ghorensis*, *G. tibanica* and *G. rufa* (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 25, 223-236.

In this work, Matthias Geiger of the Zoological Research Museum Alexander Koenig, Bonn, Germany, extracted the DNA and then provided assistance in the analysis of genomic sequences. The candidate completed all hypothesis setting and subsequent analytical interpretations.

Chapter 4 was published and was written in collaboration with Robert Britton as:

Hamidan, N. and Britton, J. R., 2015. A revised account of the geographical distribution of the endangered freshwater fish *Garra ghorensis* in Jordan and implications for conservation. *Jordan Journal of Natural History*, 2, 33-44

Chapter 5 was published and was written in collaboration with Robert Britton as:

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In this work, the stable isotope analysis was performed by Cornell Isotope Laboratory for analysis (Cornell University, New York, USA).

Chapter 1. General Introduction

1.1 Research Overview

The overall aim of this research was to assess the ecology and conservation threats to the endangered cyprinid fish *Garra ghorensis* through analysis of a range of biogeographic and ecological indicators. This fish species is endemic to the southern Dead Sea basin in the Middle East, including Jordan, the country of study. The research covers their phylogeography, contemporary distribution, life history traits and trophic ecology, with this introduction chapter providing the rationale and context for the research. The chapter concludes with the research aims, objectives and thesis structure.

1.2 Freshwater fish fauna of Jordan

The freshwater fish fauna of Jordan is relatively diverse due to its derivation from five different zoological affinities, the Palaearctic, Indoasiatic, Afrotropical, Thehys relict and Mediterranean. This diversity has attracted considerable scientific interest in the past, resulting in several studies of the fishes of the Levant (an Eastern Mediterranean area including Jordan), with authors such as Hasselquist (1757), Heckel (1843), and Tristram (1884) providing detailed accounts of the fishes of Palestine. Trewavas (1942) reviewed the cichlids of Palestine, including those of the River Jordan and Yarmouk. More recent works provided the first comprehensive taxonomical studies of Jordanian freshwater fishes, with Krupp and Schneider (1989) describing 25 freshwater fishes in the Jordan and Yarmouk river basins, of which 12 were endemic to the region, including *G. ghorensis* of the family Cyprinidae (Figure 1) and the Azraq killifish *Aphanius sirhani*. They also listed 27 introduced fishes, although few other details were provided on these non-native species.



Figure 1 *Garra ghorensis*, the focal fish of the study (© Mr Koji Kawai).

More recently, studies coordinated by the Royal Society for the Conservation of Nature (RSCN), Jordan, provided species accounts of Jordanian freshwater fishes derived from field visits completed between 2001 and 2004, and in 2014 (Hamidan, 2004, 2014). Across a series of water bodies, sampling revealed 15 fish species present belonging to 6 families and 12 genera. Similar to Krupp and Schneider (1989), the work emphasised the presence of a number of endemic fishes in these locations, including *G. ghorensis*, but also noted differences in their community composition, and population abundance and structure, across the range of waters sampled. When compared to local factors that may be constraining these fishes, threats of introduced species, habitat loss, and unsustainable water abstraction and utilisation were identified as potential major issues. However, how these factors might have been impinging on the fish populations was not ascertained. Nevertheless, these potential threats to the endemic fish fauna of Jordan were sufficient for Krupp and Schneider (1989) to suggest that unsustainable water utilisation was of particular concern for the status of the endemic *A. sirhani*. Hamidan (2004) concluded his work by saying that the combination of habitat loss and introduced species appeared to be

the most critical threats to the status of the endemic fishes, including *G. ghorensis*. This is consistent with work by numerous authors that assert that the global threat to native biodiversity posed by alien species and habitat loss is high (e.g. Marchetti et al., 2004; Clavero and Garcia-Berthou, 2006; Olden et al., 2006, Mantyka et al., 2012, Galiana et al., 2014, Walsh et al., 2016).

1.3 Fish species declines and status in Jordan, including *Garra ghorensis*

Within the freshwater fish fauna of Jordan, observed declines in the population status of a number of species are apparent. For example, Weissenbacher and Zornig (2000) assessed the Azraq Killifish *A. sirhani* as being "at the edge of extinction", resulting in the Royal Society for the Conservation of Nature (RSCN) initiating a long-term conservation programme designed to restore their populations back to their former levels. This commenced in 2000 and after starting from a stock of only 40 individual fish collected from Azraq wetland and thought to be of the last remaining individuals in the wild population, the programme has been sufficiently successful that their populations are completely restored, representing a conservation success (Soorae, 2008). This work demonstrated the conservation benefit that can accrue for threatened species in Jordan following restoration programmes that are based on data gained on the biology and ecology of the species concerned.

In 2006, the IUCN Red List classified *G. ghorensis* as being critically endangered due to its limited distribution range (Figure 2). This evaluation was made by international ichthyologists who were not fully aware about the freshwater fish studies completed in Jordan in recent years. However, in 2014, and based on field research it was reduced to 'endangered' (Freyhof, 2014) due to the high but disturbed area of occupancy the species occurred in its distribution range.

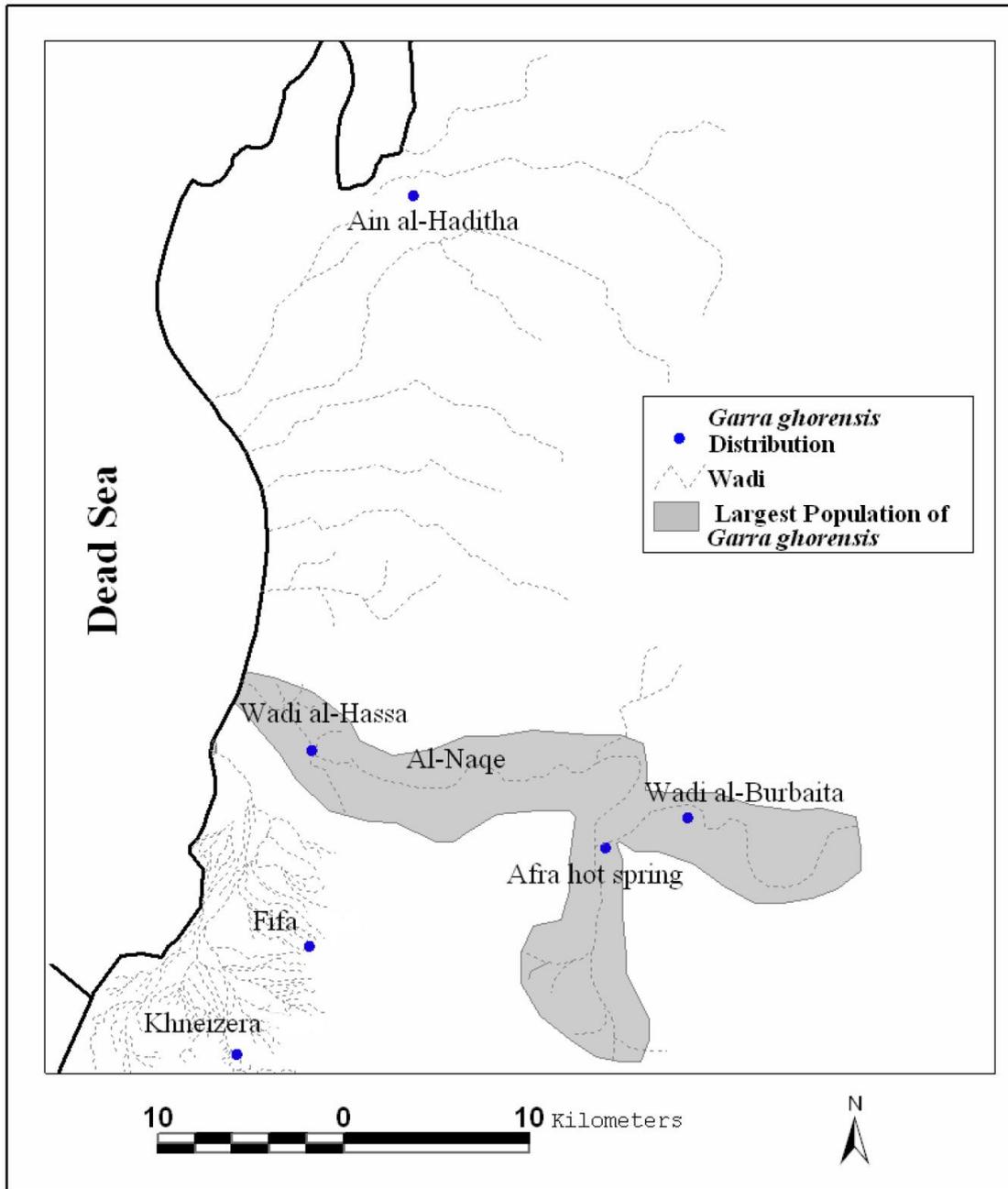


Figure 2 The known distribution of *Garra ghorensis* as determined prior to this study, recorded and published by Hamidan and Mir, 2003.

The natural range of the species is currently restricted to the springs and ‘wadis’ of the southern Dead Sea area (south of Mujib River to Wadi Khneizerah). Note a wadi (plural: wadis) refers to a stream in a valley or gully in this region and is a term used throughout the thesis. Until the 1980s this endemic species also occurred in the western side of the

Dead Sea basin, but due to the destruction of its natural habitat, it is no longer found there (Goren & Ortal 1999). In addition to the habitat destruction, at the western side of the basin, the synergistic effects of the introduced mosquito fish *Gambusia holbrooki* was noticed as bio-controllers in most of the water bodies at the western side of the previous distribution range of *G. ghorensis* (IUCN, 2006).

The impact of invasive *Gambusia* fishes is well documented around the world, and it can be summarised thus. Direct predation on native, competitive exclusion from food resources and habitat resulting in displacement of native fishes from optimal niches, and aggressive interactions in confined environments (Pyke 2008). Implications include reduced condition of native fishes, increased susceptibility of individuals to secondary infection through damage to skin and fins (i.e. via fin-nipping), and mortality-/competitive interference-driven reductions in population size and distribution (Macdonald and Tonkin, 2008). Allied with habitat loss this leads the populations of *G. ghorensis* to become extinct in the western side of the Dead Sea basin. In Jordan specifically, their rate of decline has yet to be fully quantified but it is estimated that the population has declined by at least 50 %, with their area of occupancy less than 10 km², and their habitats now severely fragmented (IUCN, 2006).

There is, however, little known about their population ecology and so although there is a strong desire to expand the *A. sirhani* conservation programme into *G. ghorensis*, there are presently no ecological or biological data on which this can be based. A baseline study, completed in 2003, initiated this process of identifying the major ecological threats to their status (Hamidan and Mir, 2003). These threats appeared to be primarily related to disturbance to habitats, such as severe modifications resulting from river damming and /or water abstraction for agriculture. The threat of alien species was also identified and

although this has yet to be quantified fully, an introduced cichlid fish (*Oreochromis aureus*) was recorded in Ein Al-Haditha the type locality where *G. ghorensis* was present.

The early descriptions of *G. ghorensis* described it as a subspecies of *Garra tibanica* (Krupp, 1982), an Arabian species closely related to the African *Garra quadrimaculata* (Stiassny and Getahun 2007). Krupp (1982) identified the *Garra* populations from the northern Dead Sea basin as *Garra rufa* and postulated that the southern Dead Sea basin was colonised by the *Garra* genus from the Arabian Peninsula / Saudi Arabia, rather than from the northern Dead Sea basin (Krupp, 1983). Conversely, Goren and Ortal (1999) and Gorshkova et al. (2012) argued against this hypothesis and postulated that *G. ghorensis* has close relationships to the other *Garra* species in the Mediterranean and Mesopotamia.

1.4 *Garra ghorensis*: current state of biological and ecological knowledge

After being described by Krupp (1982), and reviewed by Al-Absy and Mir (1986), and Krupp and Schneider (1989), no updates on the status or knowledge about *G. ghorensis* regard to its distribution or life history traits were available. Consequently, Hamidan and Mir (2003) and Hamidan (2004) provided some update on the conservation status of *G. ghorensis* in Jordan. However, these data remain limited. For example, Hamidan and Mir (2003) provided similar information to Al-Absy and Mir (1986) regarding the wide temperature range where *G. ghorensis* exists (approximately 8 to 34 C°, personal observation). Other than Krupp and Schneider (1989), however, there is no information available on their biology and ecology. Krupp and Schneider (1989) summarised the species as being fluvial, preferring fast flowing streams, with populations having some adaptation to high water temperature. They identified that the species' reproductive period was in late spring and early summer when the fish spawn in groups in the open water, eggs and sperms are released in open water, and eggs sink quite quickly into

gravel. Consequently, there remain considerable knowledge gaps for this species in many aspects of their autecology and biology. As a fish of arid regions, aspects of their ecology, population threats and conservation might be informed by fishes from similar regions elsewhere in the world, i.e. desert fishes.

1.5 Conservation of desert fishes

In the wider context, the conservation of desert fishes has received research interest due to the harsh conditions of desert habitats, and the dynamic and unpredicted changes that occur in these habitats in relation to the arid conditions and how endemic fish in particular adapt for such changes. Moreover, many freshwaters in desert areas are highly exploited for water abstraction and suffer from the detrimental effects of invasive species in a wide range of occupancy that potentially challenge conservation management efforts. Jordan is the world's fourth water-poorest country and thus has issues with freshwater storage for domestic, agricultural and industrial use. Jordan's water demand is 900 MCM/yr, with 75 % of this demand being within the Jordan River Basin (Hadadin, 2015). Total dam capacity in Jordan is estimated at 350 MCM, including the desert dams. There are seven dams constructed in the north and middle Jordan valley, with a total storage capacity of 270 MCM (Hadadin, 2015). There are also three dams (Wala, Mujib and Tannour) that are constructed in the southern Dead Sea basin that have a total live storage of 30 MCM. Stored water from these dams is used for livestock and groundwater recharge (Hadadin, 2015).

The major users of water in Jordan in 2007 (the last year where figures are publicly available) were agriculture (590.6 MCM/Year; 63 % of all water), domestic (301.5 MCM/Year; 32 %) and industry (48 MCM/Year; 5 %). This total of 940.1 MCM contrasts to the supply provided by dams of 158.7 MCM, i.e. 17 % of supply (Hadadin,

2015). Other sources are the ground water of Azraq aquifer in the east and, since 2014, the Disi aquifer in the south.

To deal with these continuous and expanded threats, integrated management planning approaches are often developed that balance the conservation of freshwater ecosystems with the human use of aquatic resources (Jones et al., 2003). Spatial prioritisation tools such as integrated watershed management, land use prioritisation, and participatory management of the upstream toward the sustainable use have the potential to identify cost-effective solutions for river conservation at the watershed scale (Nel et al., 2009). For example, Pool et al. (2013) applied this integrated management by adopting a hierarchical prioritisation approach in the Galia River basin of the Gila Mountains of southwestern Arizona, United States of America. In their work, the high priority areas (PAs) were identified and termed ‘preservation PAs’, characterised by high native fish richness and low non-native richness, and represent areas with traditional conservation targets. The areas with high native fish richness that also contained high numbers of non-native species was termed ‘restoration PAs’; these represent less traditional conservation targets. The results of Pool et al. (2013) showed that negative impacts associated with non-native species, such as parasitism, competition, and predation (Cucherousset and Olden, 2011), led to the notion that invaded stream reaches are less deserving of conservation and management attention than uninvaded or minimally invaded stream reaches. However, invaded areas where high or unique native species diversity still exists may be worthy of some management focus by contributing to the broader goals of integrated watershed management plans. The study concluded that despite the widespread support for the implementation of integrated river basin management to protect native aquatic species, there is still little consensus on the best method to focus management activity within freshwater landscapes.

A comprehensive investigation of the long-term fish responses and short-term solutions to flow regulation (via a dam) in dryland river basin of Bill Williams River in Arizona, United States of America, was provided by Pool et al. (2015) utilising a 30 year data set. In this work, the effect of flooding was analysed at both long term and short term levels. In the long-term, it was found that the non-native fish species have proliferated throughout the basin, with greater densities in the lower elevations. However, native species have persisted throughout most of the major river segments, but have experienced significant declines in frequency of occurrence and abundance in areas also containing high abundances of non-native species.

In response to flooding, a short-term reduction was observed in the abundance of non-native species in sites close to the dam, but the fish assemblage returned to its pre-flood composition within eight days of the event, with the exception of small-bodied fish, which sustained lower post-flood densities. The study concluded that these findings demonstrate the importance of natural flow regime on the balance of native and non-native species at the basin scale within dryland rivers, and highlighted the minimal effects on non-native fishes of short duration flood releases below dams.

The Lower Colorado River basin in the United States of America is one of the most studied rivers in terms of desert fishes, including the effects of biological invasions and impoundments. It has received a lot of research attention in recent years in relation to the fishes that are most at threat from environmental disturbances. Olden et al. (2006) summarised the three life history strategies that represent the endpoints of a triangular continuum arising from trade-offs among the three basic demographic parameters of survival, fecundity, and onset and duration of reproduction (e.g. Winemiller, 1989, Vila-Gispert et al., 2002, Kind and McFarlane, 2003). Based on Winemiller and Rose (1992),

these life history strategies are: (i) Periodic strategists: generally large-bodied fishes with late maturation, high fecundity per spawning event and low juvenile survivorship, (ii) opportunistic strategists: small-bodied fishes with early maturation, low fecundity per spawning event, and low juvenile survivorship and that typically inhabit highly disturbed and unpredictable environments, and (iii) equilibrium strategists: small to medium bodied fishes with moderate maturation age, low fecundity per spawning event, and high juvenile survivorship and that typically inhabit constant environments (Figure 3). Of these, periodic strategists are most vulnerable to extirpations caused by habitat disturbances, such as low flows, with opportunistic fishes the most resilient to disturbances. Whilst this body of work suggests the restoration of flows to the undisturbed states would allow the fish communities to recover within the habitat types they evolved in, given the level of development and importance of water to agriculture in the Colorado basin, this might be an unattainable goal (Olden et al., 2006).

Pool et al. (2010) investigated the environmental drivers of fish functional diversity and composition of the Lower Colorado River Basin. This was because the conservation efforts of freshwater require an understanding of how natural and anthropogenic factors shape the present-day biogeography of native and non-native species. This knowledge need is especially acute for imperilled native fishes in the highly modified Lower Colorado River Basin. In this study, both a taxonomic and functional approach was applied to explore how natural and human-related environmental drivers shape landscape-scale patterns of fish community composition in the study site.

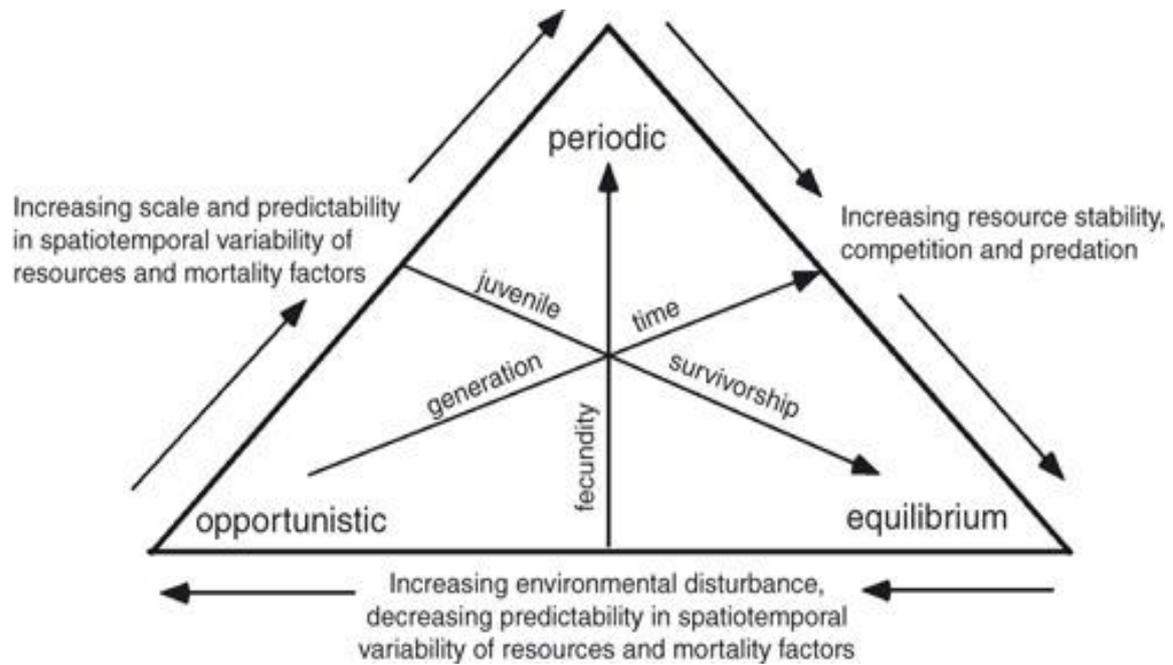


Figure 3 The life history continuum model (reproduced from Mims et al., 2010; as adapted from Winemiller (2005) and originally conceptualised in Winemiller and Rose (1992)). Inside arrows summarise fundamental trade-offs between juvenile survivorship, generation time, and fecundity that define the three end-point strategies. Outside arrows summarise how selection pressures may favour certain strategies in relation to biotic and abiotic factors (Mims et al., 2010).

The results showed that hydrologic alteration, watershed land use, and regional climate explained 30 % and 45 % of the total variation in fish community taxonomic and functional composition respectively. The study also revealed that dams have provided additional “niche opportunities” for non-native equilibrium life history strategists by introducing new reservoir habitat and modifying downstream flow and thermal regimes. By contrast, watersheds characterised by greater upstream land protection, lower dam densities, and higher variation in spring and summer precipitation supported fish communities with a strong complement of native species (opportunistic–periodic strategists). In conclusion, the study highlighted the utility of a life-history approach to

better understand the patterns and processes by which fish communities vary along environmental gradients.

Pool and Olden (2012) tested the taxonomical and functional homogenisation of the endemic fish fauna in lower Colorado River Basin. They found that fish fauna of the study site has become increasingly homogenised both taxonomically and functionally over the 20th Century. The rate of homogenisation varied substantially where range declines of native species initially caused taxonomic differentiation in the 1960s followed by marginal homogenisation observed in the 1990s in response to an influx of non-native species introductions. Conversely, the functional homogenisation of the basin was already evident in the 1950s because of the widespread introduction of non-native species sharing similar suites of biological traits. Their results were that taxonomic and functional homogenisation was positively related to the direct and indirect effects of non-native species richness. They concluded that the processes of taxonomic and functional homogenisation were highly dynamic temporally, varying in magnitude and rate of change over the 20th century.

1.6 Research aim and objectives, and thesis structure

The aim of the research is, through building on the initial work outlined on *G. ghorensis* populations to assess a series of ecological indicators (e.g. life history traits and strategies) that will enable the conservation threats to *G. ghorensis* to be assessed. These indicators will be assessed for three populations under varying levels of disturbance (a higher number of populations cannot be assessed due to their endangered status). These indicators will be supplemented by initial data on the biogeography and contemporary distribution of *G. ghorensis*. The results are discussed in relation to current and future

threats to the species, including climate change, and in relation to desert fishes more generally. The research objectives (O) and their position in the thesis are:

O1. Assess the genetic relationships between *G. ghorensis*, *G. rufa* and *G. tibanica* to identify their biogeographic origins and the phylogenetic relationships of the *Garra* genus (Chapter 3);

O2. Evaluate the current distribution of *G. ghorensis* and in relation to surveys completed in the early 2000s (Chapter 4);

O3. Quantify the age structure and somatic growth rates of *G. ghorensis* in relation to varying levels of environmental disturbance (Chapter 5);

O4. Assess the reproductive traits of *G. ghorensis* in relation to varying levels of environmental disturbance (Chapter 6); and

O5. Determine the diet, feeding interactions and trophic relationships of *G. ghorensis* in allopatry and in sympatry with a native and an invasive fish (Chapter 7).

In addition to the above, Chapter 2 provides details on the study sites of Chapters 5 to 7 in order to avoid unnecessary repetition in subsequent text. The final chapter, Chapter 8 (Discussion) provides a summary of the results of the thesis and puts them in their wider ecological and conservation contexts. Correspondingly, the structure of this thesis is:

Chapter 1: Introduction

Chapter 2: Study sites and initial sample collection

Chapter 3: The biogeographic origin and phylogenetic relationships of *Garra ghorensis* in the Southern Dead Sea basin

Chapter 4: A revised account of the geographical distribution of the endangered freshwater fish *Garra ghorensis* in Jordan and implications for conservation

Chapter 5: Age structure and somatic growth rates of *Garra ghorensis* in relation to varying levels of environmental disturbance

Chapter 6: Reproductive traits of *Garra ghorensis* in relation to varying levels of environmental disturbance

Chapter 7: Diet and trophic niche of the endangered fish *Garra ghorensis* in three Jordanian populations

Chapter 8: Discussion.

Chapter 2 Study sites, and initial samples collection

2.1 Overview

Study area is located south of the Dead Sea in Jordan. The study area comprises 14 wadi systems that are draining from east to west. These rivers used to drain and flood to the Dead Sea, where in past 20 years local and large scale impoundment took place for domestic and irrigation use, an control the water flow to the Dead Sea. Out of the 14 wadis of the study area, only seven found to have fish, where six sites are already known with their fish species content since 2002 (Hamidan and Mir, 2003).

In entirety, the spatial area covered in this work encompassed the distribution range of *G. ghorensis* as reported by Krupp (1982), Krupp and Schneider (1989), Mir (1990), Hamidan and Mir (2003) and Hamidan (2004). This area was re-surveyed in 2010 by the candidate to assure the existence of *G. ghorensis* in its known range, and to investigate any alteration to the distribution range (Chapter 4).

The study area is thus comprised the area from Ein Al-Haditha (31°17'47.74" N, 35°32'35.38"E) at the northern border and extended south to Wadi Khneizerah (30°52'53.79"N, 35°26'1.00"E) app. 50 km to the south of Ibn Hammad. It also extended east to Wadi al-Burbaitah (30°59'1.11"N, 35°40'13.71"E) at the upper tributaries of Wadi Al-Hassa (31° 0'44.95" N, 35°31'19.08"E), and from western site to rivers outlets down to the Dead Sea (Figure 4). A brief description of each site is provided in Table 3 / Chapter 4.

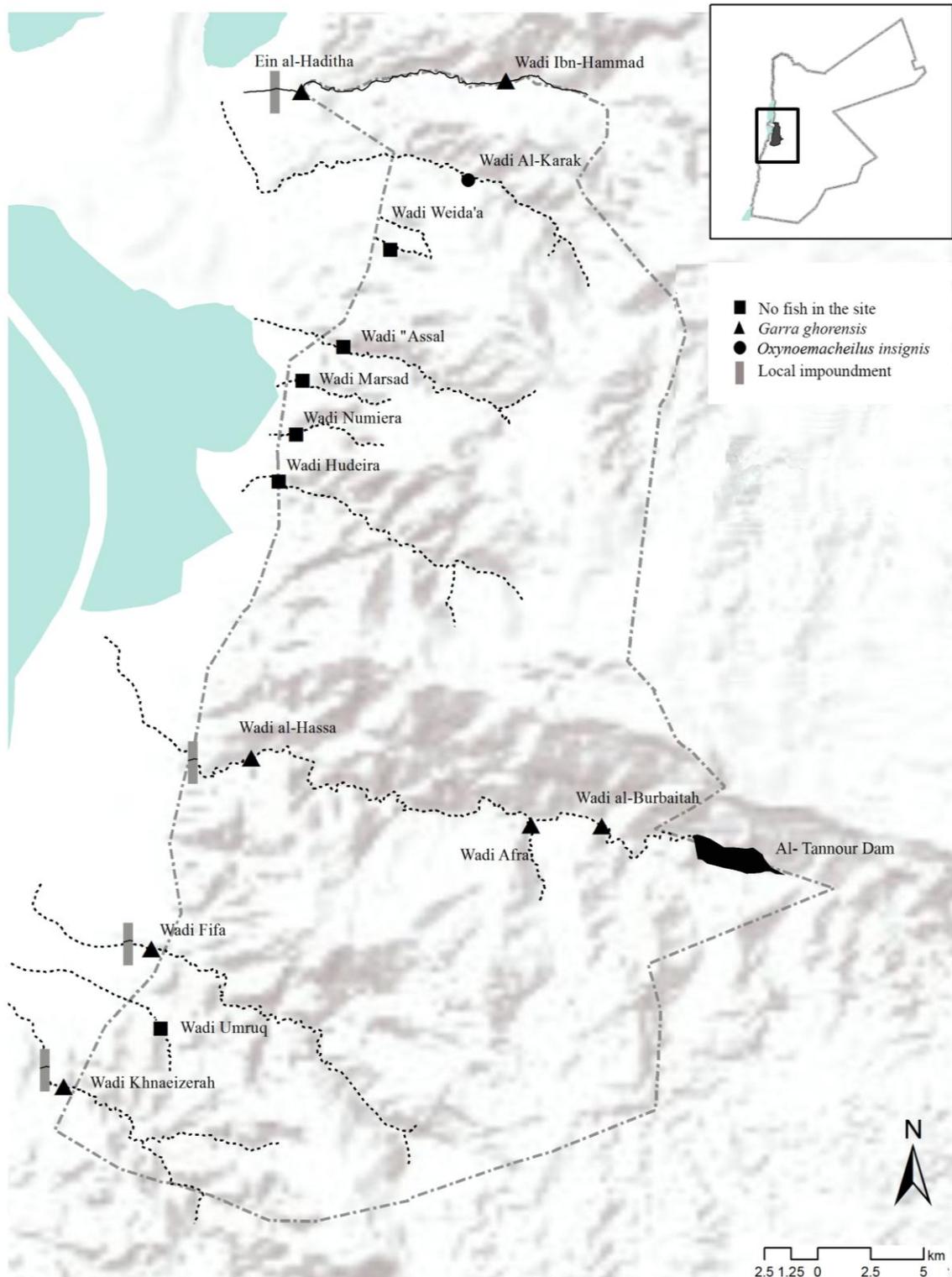


Figure 4 The study area, showing the large scale Dam Al- Tannour, local impoundments, and the current distribution range of *G. ghorensis*.

2.2 Sampling sites

The three populations of *G. ghorensis* used in the study were sampled from the sites below (Figure 5):

- Wadi-al-Burbaita (35°69'E, 30°98'N)
- Ain al-Haditha (35°54'E, 31°29'N)
- Wadi Ibn Hammad (35°38'E, 31°18'N).

These sites were chosen on the basis of their known populations of *G. ghorensis* (Hamidan, 2004) that, following survey in October 2010 (Chapter 4), were sufficiently abundant to allow some fish removals (i.e. destructive sampling would not impact their population and conservation status) and so provide fish for laboratory analyses. Wadi Ibn Hammad (hereafter referred to as site IB) is a relatively fast flowing ($0.9\text{-}1.2\text{ ms}^{-1}$) and shallow habitat in which *G. ghorensis* was the only fish present (Figure 6a). Wadi-al-Burbaita (hereafter referred to as site BR; Figure 6b) has generally lower flow rates ($0.6\text{-}0.9\text{ m s}^{-1}$) and some human disturbances through water use for domestic and agricultural uses, with *G. ghorensis* present along with the native fish *Capoeta damascina*. Ain al-Haditha (hereafter referred to site HD; Figure 6c) was the most disturbed site, with local impoundments creating sections of slower ($< 0.4\text{ m s}^{-1}$) and deeper water ($> 1\text{m}$). The invasive cichlid *Oreochromis aureus* was also present in the site, but no other fishes were recorded.

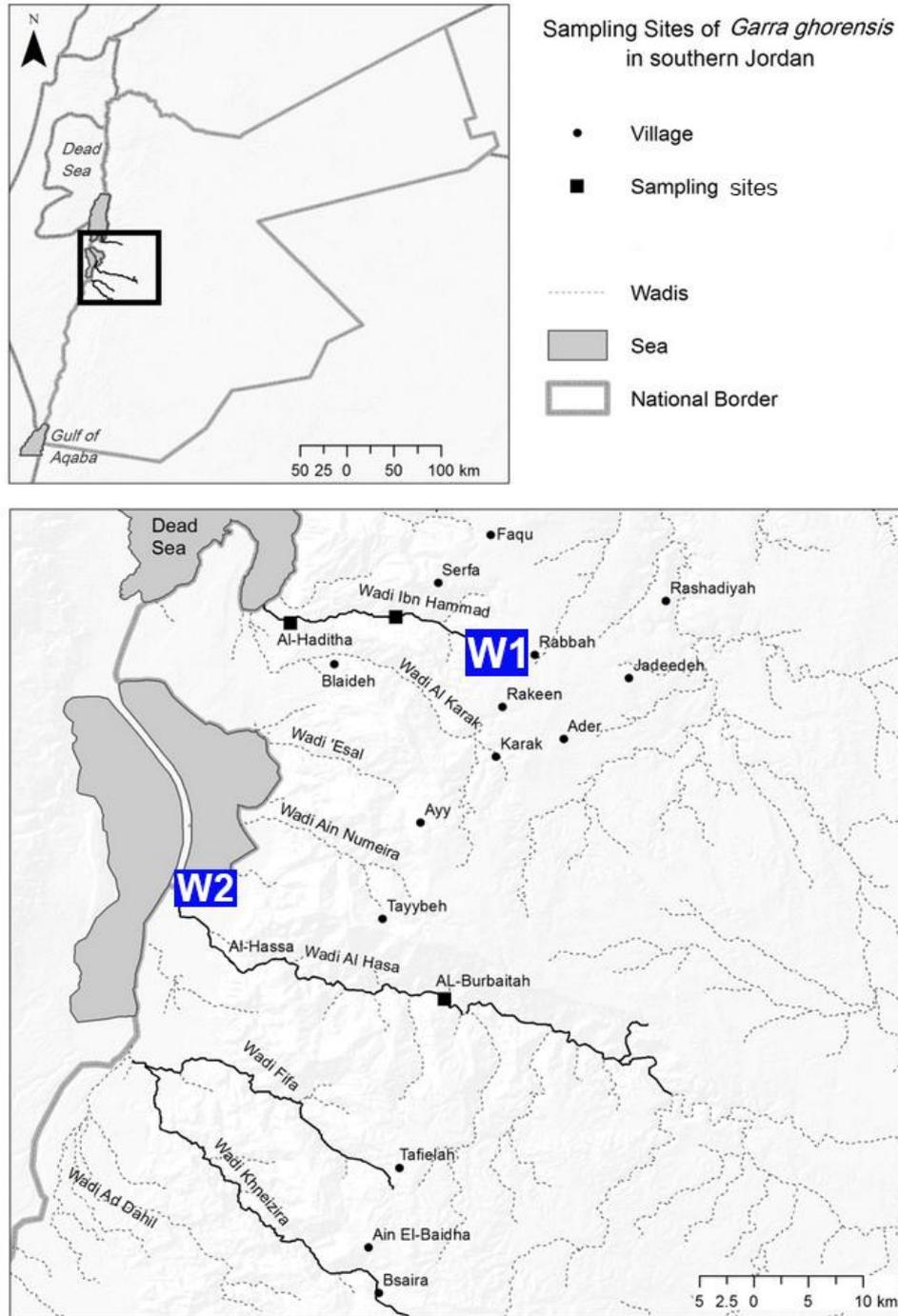


Figure 5 Location of the sites in relation to the Middle East (inset) and Jordan (main). The sampling site locations are shown on the main map by the solid black square which are Wadi Ibn Hammad (IB), Wadi-al-Burbaita (BR), and Ain al-Haditha (HD). Weather stations W1 Rabbah at elevation of 950 m above mean sea level, and W2 al Safi at -426 m below mean sea level.



a



b



c

Figure 6 Photographs of the sampling sites: (a) Wadi Ibn Hammad (IB); (b) Wadi Al-Burbaitah (BR); and (c) Ain Al-Haditha (HD).

2.2.1 Geology

In the study area to the south of the Lisan Peninsula, there are some mountains that are dissected by several wadis that run from east to west. These wadis are continuously deepening to cope with the subsidence of the base level and uplift of the source (Abed, 2000). Almost all these wadi have a base flow of fresh water depending on their location and length; e.g. Wadi Al-Hassa is the longest wadi and so has the highest base flow. Although the geology of the area is not that variable, there are some differences arising from certain structural features. The rock units cropping out within the area and as outlined briefly below, include Precambrian rocks, Ram Group, Cambrian rocks, Salib Formation, Burj Formation, Umm Ishrin Formation and lower Ordovician (Bender, 1974: Abed, 2000). In the middle and lower parts of the study area, the wadis are occupied by a sandstone regime, several hundred metres thick. However, Wadi Ibn Hammad differs from this due to the presence of a syncline, pushing most of the Ram Group sediments to the subsurface. In both wadis, a small part of Umm Ishrin Formation is cropping out, in addition to the Kurnub Group.

Quaternary deposits: The older parts of these deposits, Pleistocene in age or perhaps older (not dated), are deposited at the mouths of almost all the wadis when entering the Dead Sea basin. They consist essentially of conglomerates/gravels of varying thickness of limestone and chert rock fragments. The latest Pleistocene is best represented by the Lisan Formation which consists of varved, soft, white sediments made of alternating aragonite (calcite) with gypsum. The Lisan sediments are best seen in the Lisan Peninsula, the type area, as well as throughout the eastern part of the Dead Sea basin, including the mouths of the discussed wadis. They were deposited from the saline Lake Lisan, especially in the southern part of the present-day Dead Sea. Furthermore, the

courses of the wadis are occupied by recent, loose sediments which consist essentially of limestone and chert rock fragments ranging from sand to boulder size. Their thickness is variable and can be up to 30 m (Abed, 2000).

2.2.2 Hydrology and thermal regimes

The Dead Sea groundwater basin is located along the eastern shore of the Dead Sea. Groundwater recharge occurs primarily in the eastern highland area of the basin in the winter, and recharge is highest in the northern highlands as a result of the regional precipitation pattern (Salameh and Bannayan, 1993). Groundwater discharges through many mid- and low altitude springs towards the western part of the basin, and the ultimate discharge is to the Dead Sea, a terminal hyper-saline lake. The lowering of the Dead Sea water level during the recent decades has occurred due to the capturing of flood runoff and over-pumping of groundwater from the basin (Salameh and El-Naser, 2009). Major intermittent streams in the basin include, in the northern part, Wadi Haidan and Wadi Wala; in the central part, Wadi Mujib; and, in the southern part, and Wadi Al Hassa.

Water temperatures for the study sites were not available over the course of the study since fixing data logger in any of the three sites was not possible due to the high current and the strong seasonal flood that would remove the loggers. Thus air temperatures are used as a surrogate; the air temperatures of the weather recording stations in the vicinity of the sampling sites (W1, W2) are shown in Figure 7. These clearly demonstrate a strong seasonal pattern in air temperature that is assumed to be reflected in the thermal regimes of the study sites. At the time of the study, data available were for 2010, covering the initial four months of sampling. The mean seasonal temperatures did not significantly alter between 2010 and 2011 (personal observation).

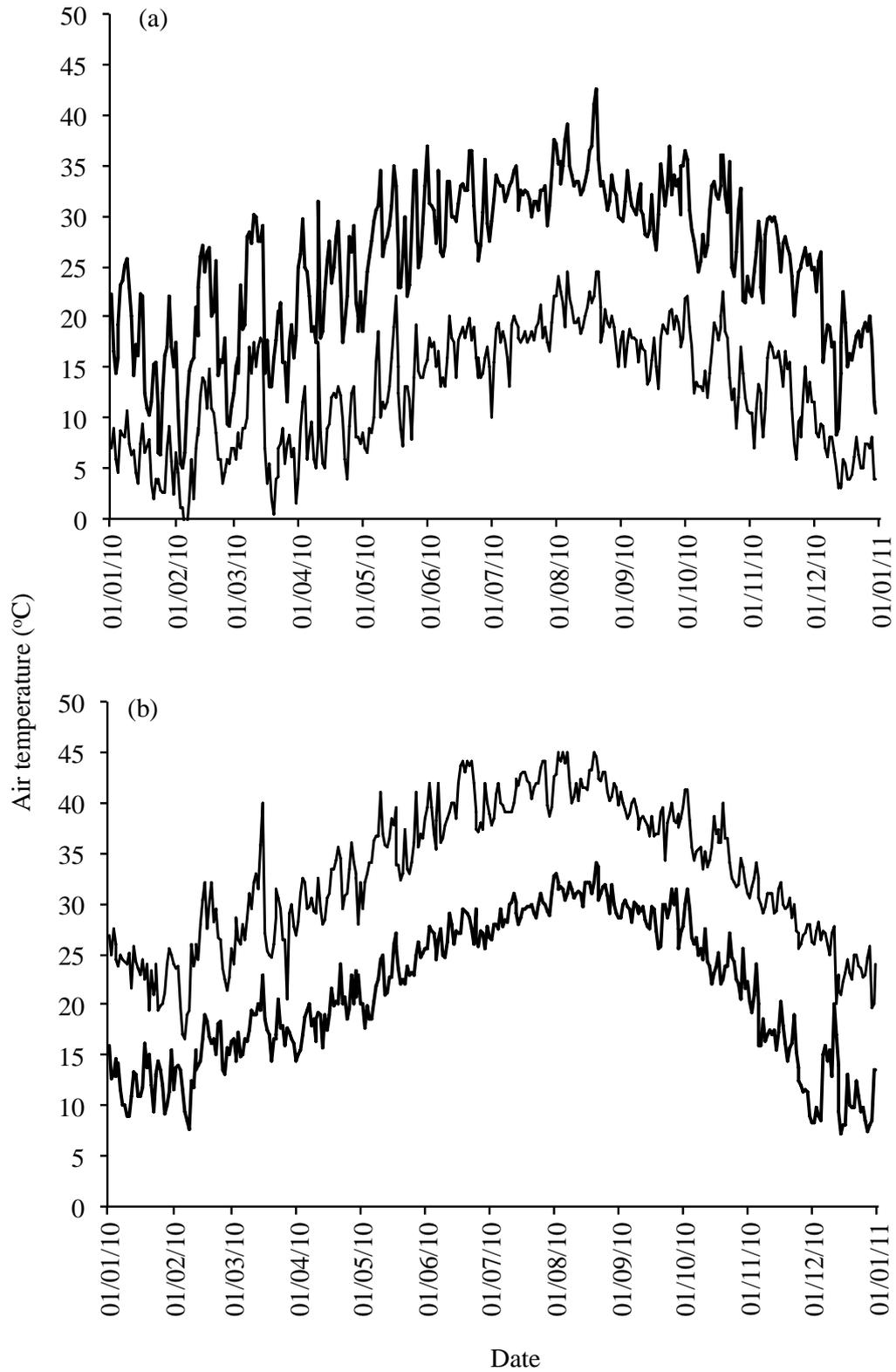


Figure 7 Daily maximum and minimum air temperature for (a) the weather station closest to site IB and BR; and (b) closest to site HD.

2.3 Fish sampling and initial data collection

The fish community of each site was sampled once per month between February 2011 and January 2012 by electric fishing. This was completed in an upstream direction for a standardised time of 30 minutes using handheld Samus 725 MP electro-fishing equipment. Where less than 15 fish were captured in this period then fishing was continued to 60 minutes to attempt to capture this number as a minimum sample size, although this was not always possible. All of the captured fish were identified in the field. In case of high numbers of fish were captured, a random sub-sample of 15 specimens was taken to the laboratory for further analysis. Permission for removing individuals was granted by licence from the Royal Society for the Conservation of Nature, Jordan. These sub-sampled fish were given an overdose of anaesthetic (overdose of anesthetic (clove oil; Soto and Burhanuddin, 1995)) before being preserved in ethanol 76%.

In the laboratory, each fish was assigned a reference number, measured using callipers (total length, fork length and standard length; to 0.1 mm), weighed (to 0.01g) and six scales removed from the area between the dorsal fin and lateral line for subsequent age and growth rate analyses (Chapter 5). These were transferred to paper envelopes for drying and long-term storage. The fish were then dissected, sexed, and their gonads were removed, weighed and then preserved in ethanol for subsequent analysis of reproductive traits (Chapter 6). The intestine was then removed and preserved in ethanol for subsequent dietary analysis; at the same time, a sample of dorsal muscle was taken for stable isotope analysis (Chapter 7). A fin clip (pelvic fin) was also taken and preserved in 95 % ethanol that was used in the genetic analyses outlined in Chapter 3.

Chapter 3. The biogeographic origin and phylogenetic relationships of *Garra ghorensis* in the Southern Dead Sea basin

This chapter has been published as a part of:

Hamidan, N., Geiger, M. and Freyhof, J., 2014. *Garra jordanica*, a new species from the Dead Sea basin with remarks on the relationship of *G. ghorensis*, *G. tibanica* and *G. rufa* (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 25, 223-236.

3.1 Overview

3.1.1. Presentation of the Chapter

This chapter is presented in two main sections. The first section (Section 3.2) outlines the biogeography of the *Garra* genus based on literature and knowledge on the importance of proto-lakes in the Pleistocene, followed by biogeographic information on the fishes in relation to the present day. This section then concludes by providing a series of hypotheses on the origin and relationships of the *Garra* genus of fishes in Jordan and the Middle East. These hypotheses are then tested in the subsequent section of the chapter (Section 3.3). This uses genetic methods to determine the phylogenetic relationships of this genus in the region and in doing so, tests the hypotheses developed in Section 3.2.

3.1.2 Summary

Phylogeography uses genetic approaches to assess the distributions of species in contemporary times in relation to the historical processes that might have resulted in these distributions. Providing that there has been appropriate sampling of individuals and genes, phylogeographic approaches can be used to test biogeographic hypotheses. Here, three hypotheses (H) were developed and tested on the origin of fishes of the *Garra* genus. H1 was that *G. ghorensis* was descended from *G. tibancia* in the Arabian Peninsula; H2 was contrary to this, stating *G. ghorensis* shared a common ancestor with *G. rufa*, indicating dispersal from the Mediterranean and Mesopotamia; and H3 stated that *Garra* fishes from the northern Dead Sea basin represented a different lineage than *G. rufa* from Mesopotamia and so represent a species yet to be described. The phylogenetic tree built from mitochondrial DNA sequences taken from over 20 species of the *Garra* genus rejected H1, but was consistent with H2, that the biogeographic origin of *G.*

ghorensis was the basins of the Mediterranean and Persian/Arabian Gulf basins. The tree also was consistent with H3, suggesting that the *Garra* fish of this part of the basin could be called a new species, *Garra jordanica*. These phylogenetic analyses thus help explain the biogeography, ancestral populations and dispersal of *G. ghorensis*, and so also have utility for interpreting patterns in their contemporary distribution.

3.2 Introduction

3.2.1 Phylogeography

Most species have sufficient population genetic structure to enable their interpretation in geographic and chronological contexts (Beheregaray, 2008). Thus, phylogeography uses approaches that assess the distributions of species in contemporary times in relation to the historical processes that might have resulted in these distributions. Providing that there has been appropriate sampling of individuals and genes, phylogeographic approaches can be used to test biogeographic hypotheses, describe the evolution of reproductive isolation of population units, and infer processes underlying the origin, distribution and maintenance of biodiversity (Beheregaray, 2008). As the structure of population genealogies tend to be strongly influenced demographic history, analyses can also infer temporal changes in the physical and biotic environment of a population from data collected in the present (Feliner, 2014; Mossop et al., 2015). Thus, it can be applied to understand speciation processes (e.g. Hewitt, 2001; Kohn, 2005) and historical biogeography (Avice, 2000; Riddle and Hafner, 2006), as well as other processes including palaeoecology and conservation biology (Beheregaray, 2008).

3.2.2 *Phylogeography of the Cyprinidae family*

The family Cyprinidae is the second largest fish family in the world and one of the most widespread in freshwater, with natural ranges encompassing all continents other than South America and Australia (Durand et al., 2002). Cyprinid fishes are primarily freshwater species. So, given that they are unable to cross stretches of saline water, their distributions should reflect the geological evolution of the landscape (Durand et al., 2002; Mesquita et al., 2005; Aboim et al., 2013). Molecular phylogenetic studies have already utilised the Cyprinidae to, for example, define phylogenetic links (e.g. Gilles et al., 1998) and make biogeographical inferences (e.g. Tsigenopoulos and Berrebi, 2000; Machordom and Doadrio, 2001). These have indicated that the Middle East is an important region for cyprinid biogeography due to its location; some consider it to represent either a major biogeographical crossroads (Banarescu, 1992; Coad, 1996a), or a centre of speciation (Por and Dimentman, 1985). The former has the greater support, given that fishes are capable of arriving in the region via dispersal from the south (Africa), west (Palearctic), and east (Western Asia) (Banarescu, 1992). The region is also often considered as a major zoogeographical interchange area and, correspondingly, there are difficulties in identifying the adjacent region(s) that most influenced its ichthyofauna (Durand et al., 2002). Some argue it belongs to the Palearctic region (e.g. Coad, 1996a) whilst others suggest it is a sub-region of the Oriental region (Banarescu, 1992). Irrespective, it means there is scope for the origins of Cyprinid fish in the region to be heavily influenced by the ichthyofauna of fish in neighbouring regions, with this providing a key question in the context of their contemporary distribution (Section 3.3).

3.2.3 Historical fish biogeography in Jordan: importance of proto-lakes in the Pleistocene

The general presence and/ or absence of the fish species in Jordan and the surrounding areas, and the specific presence of species of the *Garra* genus, is potentially explained biogeographically by formation and presence of proto-lakes in the Dead Sea–Jordan Valley region during the Pleistocene. Of particular interest are the Samra and Lisan Lakes (Figure 8).

The Samra Lake, named after the ruins of Samra (Kherbet Samra, some 6 km NNE of Jericho), was present 135-70/65 Ka (thousand years) ago. It preceded the Lisan Lake by 70/65-16/15 Ka and so its sediments underlie the Lisan sediments. Both lakes belong to the uppermost Pleistocene. The Samra Lake level fluctuated between 320-380 m below mean sea level (BMSL), meaning it was about 200 m lower than the Lisan Lake level of around 170 m BMSL, but higher than present day Dead Sea level of 430 BMSL (Bartov et al., 2003). Lisan Lake occupied a larger area within the Jordan Valley-Dead Sea basin compared with Samra Lake, especially in an east-west direction. In addition, and because the Samra deposits are overlain by the widespread Lisan sediments, the former deposits are mostly in the subsurface; i.e. they are not as conspicuous as the Lisan sediments. Recent works (e.g. Bartov et al., 2003; Waldmann et al., 2009; Neugebauer et al., 2016) have identified the Samra Lake deposits from outcrops and boreholes in the Jordan Valley, the Dead Sea basin and also further south of these. This simply means that the Samra Lake had occupied the same area as the Lisan Lake in a north-south direction; i.e. along the rift valley system

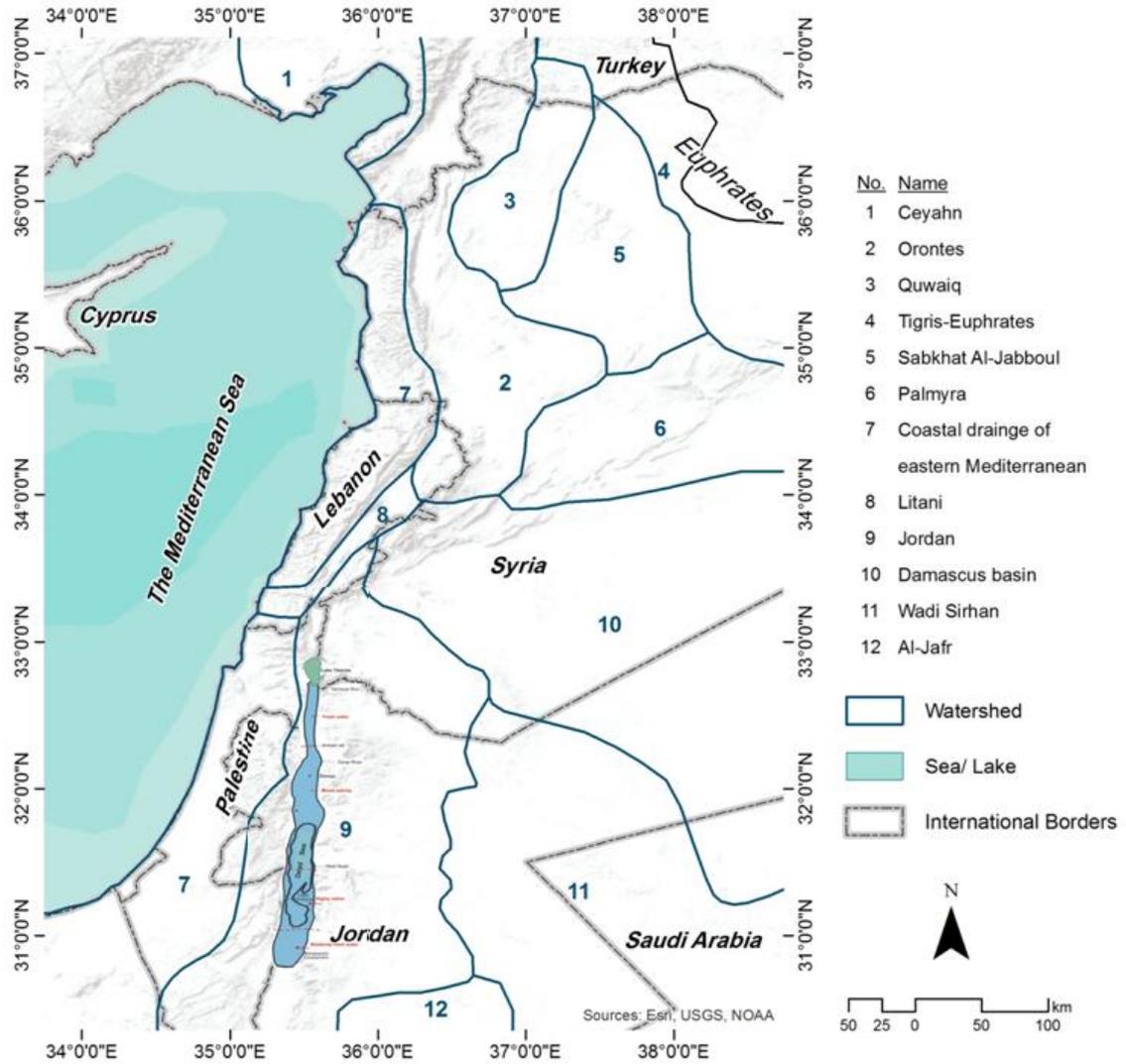


Figure 8 Lisan Lake location in relation to the surrounding watersheds.

The Samra Lake sediments consist of conglomerates, limestones and marls with no evaporates, indicating a fresh water lake (Abed, 1985; Abed and Yaghan, 2000). Consequently, fish were most likely present in the lake and in wadis that discharged into it, including Wadi Mujib, one of the oldest wadis at the eastern site of the Rift Valley (Bareberi et al., 1979; Abed, 2000). Lisan Lake developed in place of Samra lakes after further geological and glacial changes (Kaufman, 1971; Abu Ghazleh, 2011) and finally extended from the present Lake Tiberias in the north to Wadi Khneizereh in the south of the Dead Sea (Figure 8).

Today, Lisan Lake sediments are encountered across the 220 km length of the lake (Abu Ghazleh, 2011), with this 'Lisan formation' consisting of deposits with a typical thickness of 30 m consisting of authigenic aragonite and gypsum layers that alternate with thin silt and sand detritus layers (Katz et al., 1977; Stein, 2001; Begin et al., 2004). For most of its history (70,000–28,000 years B.P.), the lake maintained a stable water level of -280 ± 20 m above sea level (ASL), with two main level drops at 67–63 and 47–43 ka (Bartov et al., 2003; Haase-Schramm et al., 2004). At 28,000 years B.P., the lake level rose and at about 26,000 years B.P., the lake reached a maximum level of -165 m ASL (Bartov et al., 2003), standing more than 200 m higher than the present Dead Sea (Figure 9). At that stage, Lake Lisan coalesced with the ancient Lake Tiberias (Hazan, 2001), becoming 240 km long and 7 to 15 km wide (Neev and Emery, 1967). At 14,000 years B.P. the lake receded to -280 m, and the recession continued (Bartov et al., 2003; Stein, 2001).

Of biogeographic importance is that the salinity of Lisan Lake, as inferred from its sediments, was variable (Figure 9), due to the area near Al-Karn in the Jordan Valley being tectonically narrowed and elevated that produced a natural barrier to the water flow southwards (Abed, 1985, Begin et al., 2004). Consequently, north of Al-Karn, the lake was fresh, indicated by abundant freshwater diatoms with no evaporite minerals in the deposits, whereas it was saline in all parts near Al-Karn and the Lisan Peninsula, as revealed by gypsum deposits (Abed, 1983, 1985) (Figure 9). Summer salinity levels were likely to have been high (at least 100 g l^{-1}) (Abed, 1985, Begin et al., 2004), preventing fish species from surviving and thus influencing the contemporary biogeographic fish distribution, as explored in Chapter 4.

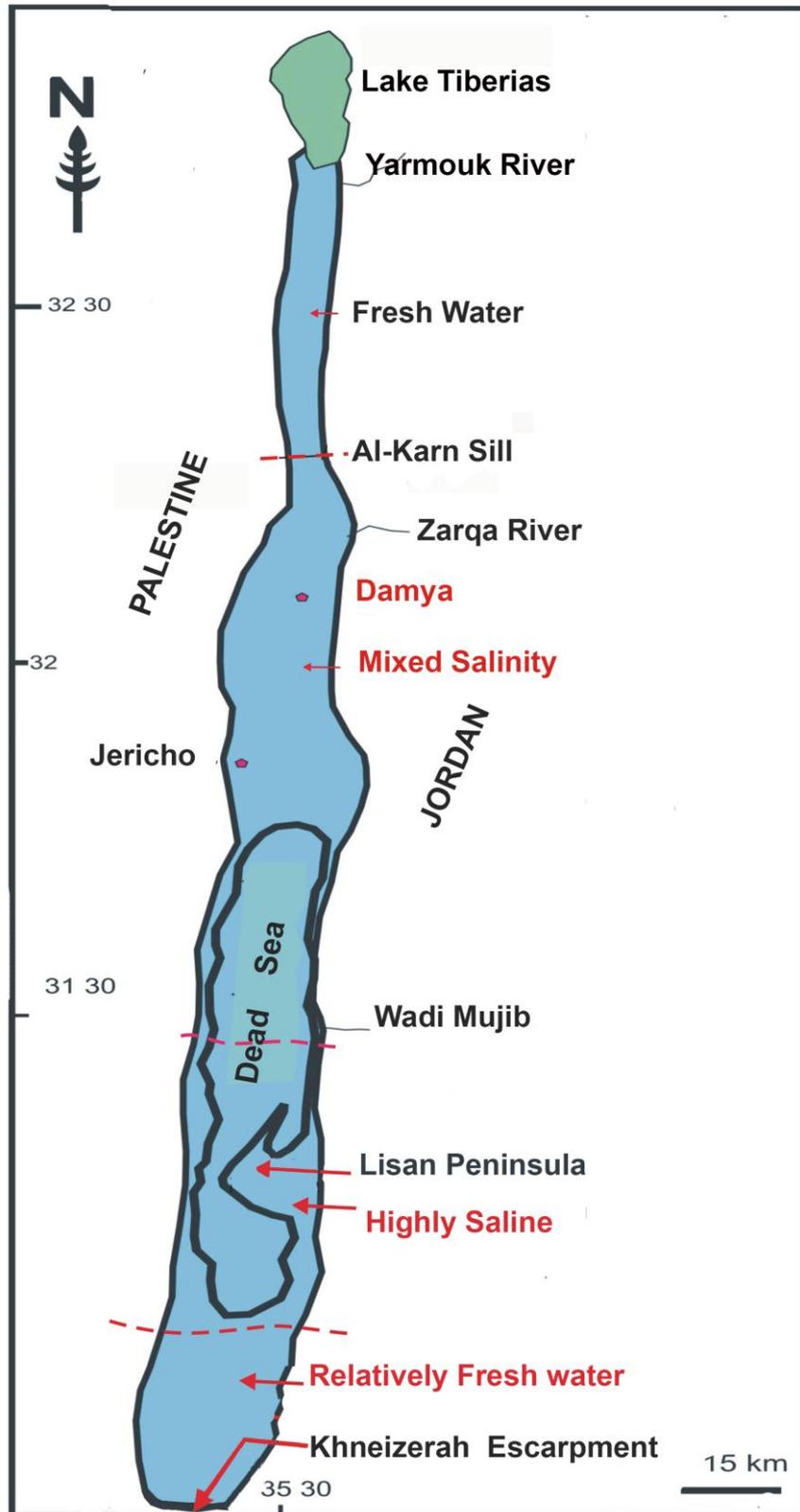


Figure 9 Different levels of salinities in Lisan Lake (Abed 1985).

3.2.4 Contemporary knowledge on the biogeography of the *Garra* genus in Jordan

Cyprinid fishes of the genus *Garra* are widely distributed across subtropical and tropical Asia, the Middle East, and Africa (Menon, 1964). In the Mediterranean basin, ten species are recognised by Geiger et al. (2014), of which only three have a mental adhesive disc (Figure 10). The adhesive disc is used in feeding where the mouth pads sticks to the substrate and the disc scratches the algae. The disc and its surrounding structure is also used for holding position against the high flow and strong flood. These *Garra* with mental adhesive disc are: *Garra variabilis*, distributed in the Asi and Nahr al Kabir drainages in Syria, *G. ghorensis*, distributed in the southern tributaries of the Dead Sea basin (Hamidan and Mir, 2003), and *G. rufa*, found in the Seyhan River in Turkey and south to the northern tributaries of the Dead Sea basin (Krupp, 1985). In addition to these three species, *Garra jordanica* was recently described (Hamidan, et al., 2014) with a well developed mental disc, that increased the number of *Garra* species with mental disc identified by Geiger et al. (2014) to four (Figure 11). These fishes with a mental disc share very similar morphological features and thus are interpreted as being closely related, especially as the mental disc was considered as diagnostic for the genus *Garra* (Menon, 1964). In addition, the data presented by Geiger et al. (2014) suggested that the *Garra* populations from the region in the northern Dead Sea basin represented a different lineage than *G. rufa* from Mesopotamia, and might represent a species yet to be described. Whilst *G. ghorensis* is endemic to the southern Dead Sea basin, *G. variabilis* and *G. rufa* occur in the Persian/Arabian Gulf basin, where the former is widespread in the upper Qweik, Euphrates and Tigris drainages, and the latter has a wider distribution range occurring all over from the Qweik and the Euphrates and Tigris drainage south to the Mond River, which flows into the Persian/Arabian Gulf in Iran.



Figure 10 The adhesive disc of *Garra jordanica*, located on the underside of the fish. Source: Hamidan et al. (2014).

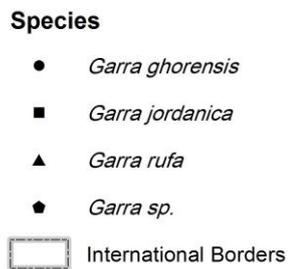
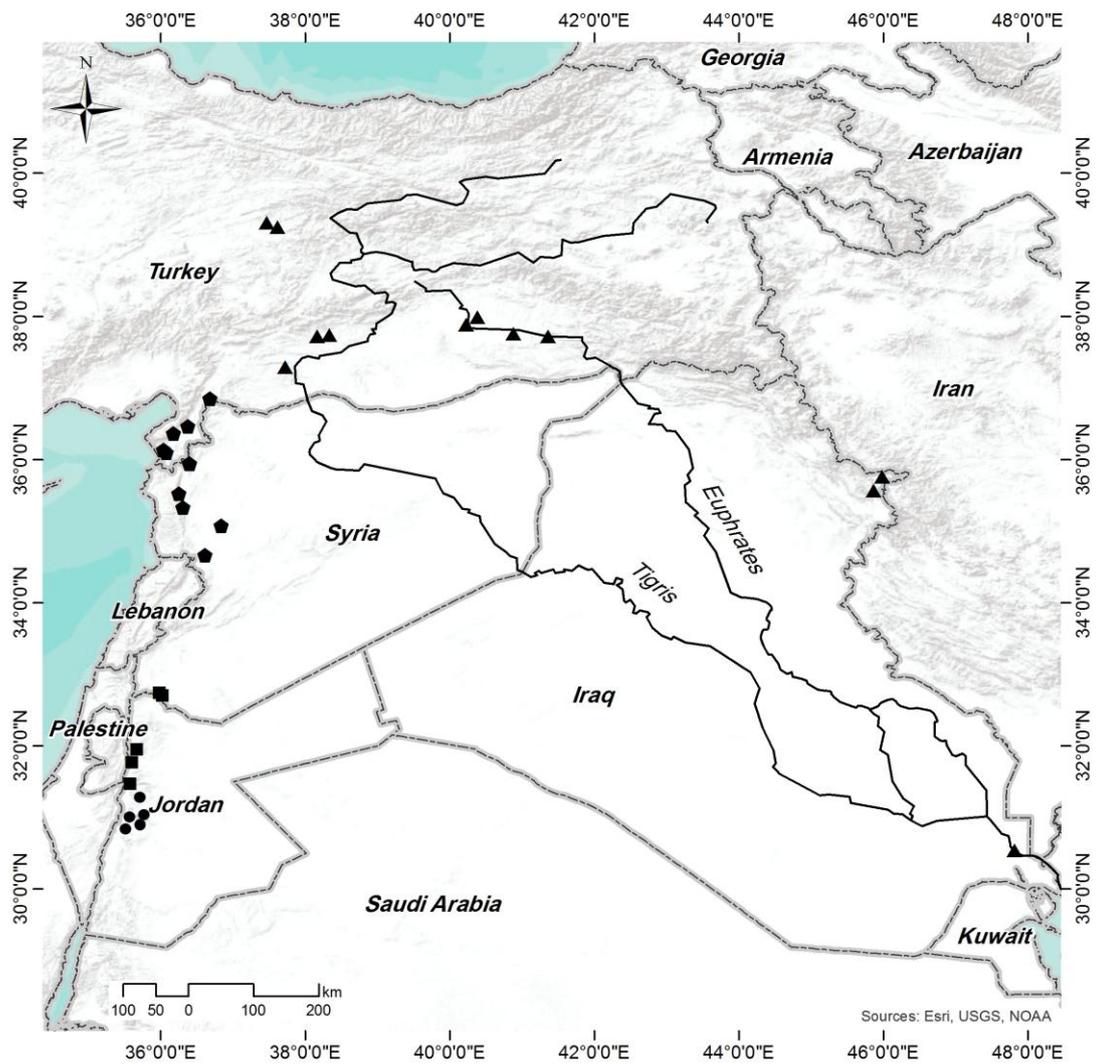


Figure 11 The distribution range of the four *Garra* species with close association with the hypotheses (Hamidan et al. 2014).

Garra ghorensis was described by Krupp (1982) as a subspecies of *G. tibanica*, an Arabian species closely related to, or even identical with, the African species *Garra quadrimaculata* (Stiassny and Getahun 2007). Krupp (1982) identified the *Garra* populations from the northern Dead Sea basin as *G. rufa* and postulated that the southern Dead Sea basin was colonised by *Garra* from the Arabian Peninsula and not from the northern Dead Sea basin (Krupp, 1983) (Figure 11, 12). Goren and Ortal (1999) and Gorshkova et al. (2012) argued against this hypothesis and postulated that *G. ghorensis* has close relationships to the other *Garra* species in the Mediterranean and Mesopotamia (Figure 11, 12). To date, however, there have been no genetic studies completed on the phylogeography of *G. ghorensis* and so their biogeographic origin remains uncertain. These arguments on the origin of the *Garra* genus thus give rise to three hypotheses.

3.2.5 Biogeographic hypotheses on the origin of the Garra genus in Jordan and the Middle East for testing with phylogeography

Following Section 3.2.3 and 3.2.4, the following hypotheses (H) have been developed for testing in Section 3.3:

H1. *Garra ghorensis* descended from *G. tibancia* in the Arabian Peninsula, as per Krupp (1982);

H2. *Garra ghorensis* shares a common ancestor with *G. rufa*, indicating dispersal from the Mediterranean and Mesopotamia, as per Goren and Ortal (1999) and Gorshkova et al. (2012); and

H3. *Garra* fishes from the northern Dead Sea basin represent a different lineage than *G. rufa* from Mesopotamia and represent a species yet to be described (referred to as *Garra jordanica* here) (Hamidan et al., 2014)



Figure 12 A: *Garra ghorensis*; B: *Garra jordanica*; C: *Garra rufa* (©Yazdan Keivany); and D: *Garra tibanica*.

3.3 Assessing the phylogeography of the *Garra* genus

The rationale for this section was developed and outlined in Section 3.2. The aim of the section was to test the three hypotheses provided at the end of Section 3.2.5 using genetic approaches.

3.3.1 Material and methods

Sample collection

To determine the phylogenetic relations of *Garra* genus and enable testing of the hypotheses, a total of 62 individuals of *Garra* representing 24 species were examined (Table 1 and 2). These specimens were collected from across the Arabian Peninsula, Jordan, the Mediterranean basin, Mesopotamia and Africa. In order to better understand the phylogenetic position of the studied species, records from Genbank were added for *Garra congoensis* and *Garra ornata* from Democratic Republic of Congo (DRC), (DRC), and for *Garra waterloti* from Guinea. Specimens held by two collaborators (n = 13, 4) were also used, as well as samples collected by the candidate (n = 6 species and one subspecies) (Table 2). For these latter species, individuals were collected by using a cast net 12 mm, and handheld Samus 725 MP electro-fishing equipment. Following their capture, the fish were euthanized (over-dose of clove oil) and preserved in high grade ethanol. In the laboratory, tissue samples were taken, usually of pelvic fin tissue.. Collection of larger number of specimens was constrained by the endangered conservation status of these species like *G. ghorensis*, or the low numbers of such species in their habitats. Sampling was also limited by the tough terrain and the inaccessibility of some sites such as Wadi Fifa and Wadi Khneizerah. As for specimens that were obtained from the Breeding Centre of the Endangered Arabian Wild life Species, the Centre does

not permit more than three specimens due to their unknown status, endemic, and limited distribution of species such as *G. dunserei*.

Table 1 Number of individuals of each *Garra* species that were used for DNA analysis.

Species	Individuals analysed (n)
<i>Garra</i> sp.	2
<i>Garra culiciphaga</i>	1
<i>Garra rufa</i>	5
<i>Garra klatti</i>	2
<i>Garra kemali</i>	1
<i>Garra caudomaculata</i>	1
<i>Garra variabilis</i>	2
<i>Garra nana</i>	1
<i>Garra jordanica</i>	7
<i>Garra sauvagei</i>	1
<i>Garra ghorensis</i>	7
<i>Garra festai</i>	1
<i>Garra typhlops</i>	1
<i>Garra widdowsoni</i>	1
<i>Garra smarti</i>	3
<i>Garra cf. longipinnis</i>	2
<i>Garra dunserei</i>	3
<i>Garra sahilia</i>	7
<i>Garra buettikerii</i>	3
<i>Garra tibanica</i>	3
<i>Garra barreimiae</i>	5
<i>G. waterloti</i>	1
<i>G. congoensis</i>	1
<i>G.ornata</i>	1
Total	62

Table 2 Specimens of *Garra* species that were used for sequencing of their mitochondrial DNA, with information on their sampling site, tissue number, DNA aliquots of each sample, location, and collector. Location represents co-ordinates in decimal degree.

Species and Location	Tissue Sample No.	DNA Aliquots	Location	Collector
<i>Garra</i> sp. Orontes Turkey	331	Ex14B10	36.851983, 36.686083	Jörg Freyhof
<i>Garra</i> sp. Orontes Turkey	331	Ex14B8	36.851983, 36.686083	Jörg Freyhof
<i>Garra</i> sp. Orontes Turkey	331	Ex14B9	36.851983, 36.686083	Jörg Freyhof
<i>Garra</i> sp. Orontes Syria	1140	Ex15F6	37.950833, 36.395833	Jörg Freyhof
<i>Garra</i> sp. Orontes Syria	1140	QSCII14TIS1140-1	35.95083, 36.39583	Jörg Freyhof
<i>Garra</i> sp. Orontes Syria	1140	QSCII14TIS1140-2	35.95083, 36.39583	Jörg Freyhof
<i>Garra culiciphaga</i> Seyhan Turkey	400	Ex14H2	36.975683, 35.335417	Jörg Freyhof
<i>Garra culiciphaga</i> Seyhan Turkey	400	Ex14H3	36.975683, 35.335417	Jörg Freyhof
<i>Garra culiciphaga</i> Seyhan Turkey	400	Ex14H4	36.975683, 35.335417	Jörg Freyhof
<i>Garra rufa</i> Tigris Turkey	918	Ex54E4	37.887167, 40.229800	Jörg Freyhof
<i>Garra rufa</i> Firat Turkey	923	QSCII14TIS923-1	37.72236, 38.4479	Jörg Freyhof
<i>Garra rufa</i> Firat Turkey	923	QSCII14TIS923-2	37.72236, 38.4479	Jörg Freyhof
<i>Garra rufa</i> Firat Turkey	923	QSCII14TIS923-3	37.72233, 38.44794	Jörg Freyhof
<i>Garra klatti</i> Köprü Turkey	1104	Ex15E4	37.763617, 31.033567	Jörg Freyhof
<i>Garra klatti</i> Isikli Turkey	1074	Ex48C3	38.122767, 30.095383	Jörg Freyhof
<i>Garra kemali</i> Tuz Turkey	1076	Ex15C11	37.986133, 33.351350	Jörg Freyhof
<i>Garra kemali</i> Tuz Turkey	1076	Ex15C12	37.986133, 33.351350	Jörg Freyhof
<i>Garra caudomaculata</i>	1143	Ex18E9	37.950833,	Jörg

Species and Location	Tissue Sample No.	DNA Aliquots	Location	Collector
Orontes Syria			36.395833	Freyhof
<i>Garra variabilis</i> Orontes Syria	1159	Ex15G4	35.304980, 36.350770	Jörg Freyhof
<i>Garra variabilis</i> Orontes Syria	1159	Ex15G5	35.304980, 36.350770	Jörg Freyhof
<i>Garra variabilis</i> Orontes Syria	1168	Ex15G10	35.271667, 36.562778	Jörg Freyhof
<i>Garra variabilis</i> Orontes Syria	1168	Ex15G9	35.271667, 36.562778	Jörg Freyhof
<i>Garra nana</i> Al Tammasiyyar Syria	1182	Ex48A5	33.293611, 35.924722	Jörg Freyhof
<i>Garra nana</i> Al Tammasiyyar Syria	1182	Ex48A6	33.293611, 35.924722	Jörg Freyhof
<i>Garra jordanica</i> Jordan Syria	1186	Ex82C4	31.770556, 35.602778	Jörg Freyhof
<i>Garra jordanica</i> Jordan Syria	1186	Ex82C5	31.770556, 35.602778	Jörg Freyhof
<i>Garra jordanica</i> Jordan Syria	1238	Ex18G7	32.703333, 36.022222	Jörg Freyhof
<i>Garra jordanica</i> Jordan Syria	1206	Ex16A11	32.739167, 35.982222	Jörg Freyhof
<i>Garra sauvagei</i> Jordan Syria	1187	Ex16A5	32.738889, 36.009167	Jörg Freyhof
<i>Garra ghorensis</i> Dead Sea Jordan	1193	Ex16A6	31.296389, 35.542500	Jörg Freyhof
<i>Garra ghorensis</i> Dead Sea Jordan	1193	Ex16A7	31.296389, 35.542500	Jörg Freyhof
<i>Garra ghorensis</i> Dead Sea Jordan	1193	Ex16A8	31.296389, 35.542500	Jörg Freyhof
<i>Garra ghorensis</i> Jordan	1225	FSJF2670	30.965544, 35.6822	Jörg Freyhof
<i>Garra festai</i> Litani Lebanon	2153	Ex25F3	33.732500, 35.784444	Jörg Freyhof
<i>Garra festai</i> Litani Lebanon	2153	Ex25F4	33.732500, 35.784444	Jörg Freyhof
<i>Garra festai</i> Litani Lebanon	2153	Ex25F5	33.732500, 35.784444	Jörg Freyhof
<i>Garra typhlops</i> KaajeRu Iran	2169	WH35SC2169x2	Not available	Jörg Freyhof
<i>Garra typhlops</i> KaajeRu Iran	2169	WH35SC2169x3	Not available	Jörg Freyhof

Species and Location	Tissue Sample No.	DNA Aliquots	Location	Collector
<i>Garra rufa</i> Tigris Iraq	2234	Ex72A11	35.808889, 45.022222	Jörg Freyhof
<i>Garra rufa</i> Tigris Iraq	2234	Ex72A12	35.808889, 45.022222	Jörg Freyhof
<i>Garra rufa</i> Tigris Iraq	2234	Ex72B1	35.808889, 45.022222	Jörg Freyhof
<i>Garra widdowsoni</i> Euphrates Iraq	2301	Ex72B7	34.066667, 42.400000	Jörg Freyhof
<i>Garra widdowsoni</i> Euphrates Iraq	2301	Ex72B8	34.066667, 42.400000	Jörg Freyhof
<i>Garra rufa</i> Euphrates Turkey	2414	Ex63H5	Not available	Jörg Freyhof
<i>Garra rufa</i> Euphrates Turkey	2414	Ex63H6	Not available	Jörg Freyhof
<i>Garra rufa</i> Euphrates Turkey	2414	Ex63H7	Not available	Jörg Freyhof
<i>Garra rufa</i> Shatt al-Arab Iraq	2480	Ex89A2	30.539517, 47.831181	Jörg Freyhof
<i>Garra smarti</i> Hasaq Oman	cn5874	cn5874	17.434936, 55.227808	Nashat Hamidan
<i>Garra smarti</i> Hasaq Oman	cn5893	cn5893	17.434936, 55.227808	Nashat Hamidan
<i>Garra cf. longipinnis</i> Jabal al Akhdar Oman	cn5897a	cn5897	Not available	Nashat Hamidan
<i>Garra cf. longipinnis</i> Jabal al Akhdar Oman	cn5897b	cn5897b	Not available	Nashat Hamidan
<i>Garra smarti</i> Hasaq Oman	cn773	cn773	Not available	Nashat Hamidan
<i>Garra dunserei</i> Dhofar Oman	CN7766	CN7766	17.113164, 54.560464	Nashat Hamidan
<i>Garra dunserei</i> Dhofar Oman	CN7769	CN7769	17.113164, 54.560464	Nashat Hamidan
<i>Garra dunserei</i> Dhofar Oman	CN7771	CN7771	17.113164, 54.560464	Nashat Hamidan
<i>Garra sahilia</i> Sanaa Yemen	cn789	cn789	15.354,44.206	Nashat Hamidan
<i>Garra sahilia</i> Sanaa Yemen	cn872	cn872	15.354,44.206	Nashat Hamidan
<i>Garra ghorensis</i> Burbaita Jordan	NHJO-001	Ex91H5	30.98377, 35.66992	Nashat Hamidan
<i>Garra ghorensis</i> Burbaita Jordan	NHJO-002	Ex91H4	30.98377, 35.66992	Nashat Hamidan

Species and Location	Tissue Sample No.	DNA Aliquots	Location	Collector
<i>Garra ghorensis</i> Burbaita Jordan	NHJO-004	Ex91H6	30.98377, 35.66992	Nashat Hamidan
<i>Garra ghorensis</i> Ibn-Hammad Jordan	NHJO-009	Ex91H3	31.301397, 35.62984	Nashat Hamidan
<i>Garra ghorensis</i> Fifa Jordan	NHJO-027	Ex91G12	30.931328, 35.481166	Nashat Hamidan
<i>Garra jordanica</i> Dead Sea Jordan	NHJO-041	Ex91E12	31.456356, 35.585746	Nashat Hamidan
<i>Garra jordanica</i> Dead Sea Jordan	NHJO-042	Ex91E10	31.456356, 35.585746	Nashat Hamidan
<i>Garra jordanica</i> Dead Sea Jordan	NHJO-043	Ex91E11	31.456356, 35.585746	Nashat Hamidan
<i>Garra jordanica</i> Dead Sea Jordan	NHJO-045	Ex91F1	31.456356, 35.585746	Nashat Hamidan
<i>Garra sahilia</i> div Wadis SA	NHKSA-012	Ex91G3	18.776082, 41.987773	Nashat Hamidan
<i>Garra sahilia</i> div Wadis SA	NHKSA-014	Ex91G4	18.776082, 41.987773	Nashat Hamidan
<i>Garra sahilia</i> div Wadis SA	NHKSA-015	Ex91G5	18.776082, 41.987773	Nashat Hamidan
<i>Garra sahilia</i> div Wadis SA	NHKSA-016	Ex91G6	18.776082, 41.987773	Nashat Hamidan
<i>Garra buettikerii</i> div Wadis SA	NHKSA-020	Ex91F3	20.589190, 41.289086	Nashat Hamidan
<i>Garra buettikerii</i> div Wadis SA	NHKSA-021	Ex91F5	20.589190, 41.289086	Nashat Hamidan
<i>Garra buettikerii</i> div Wadis SA	NHKSA-022	Ex91F4	20.540388, 41.286326	Nashat Hamidan
<i>Garra buettikerii</i> div Wadis SA	NHKSA-024	Ex91F6	20.762300, 41.231388	Nashat Hamidan
<i>Garra sahilia</i> div Wadis SA	NHKSA-026	Ex91G7	25.74726, 39.260362	Nashat Hamidan
<i>Garra tibanica</i> Wadi damad SA	NHKSA-027	Ex91G8	25.747262, 39.260362	Nashat Hamidan
<i>Garra tibanica</i> Wadi damad SA	NHKSA-028	Ex91G9	25.74726, 39.260362	Nashat Hamidan
<i>Garra tibanica</i> Wadi damad SA	NHKSA-031	Ex91G10	25.7472623, 39.26036	Nashat Hamidan
<i>Garra barreimiae</i> Wuray UAE	NHUAE-001	Ex91F7	25.398400, 56.269537	Nashat Hamidan
<i>Garra barreimiae</i> Wuray UAE	NHUAE-002	Ex91F8	25.398400, 56.269537	Nashat Hamidan

Species and Location	Tissue Sample No.	DNA Aliquots	Location	Collector
<i>Garra barreimiae</i> Wuray UAE	NH UAE-006	Ex91F10	25.398400, 56.26953	Nashat Hamidan
<i>Garra barreimiae</i> Shawkah UAE	NH UAE-011	Ex91F11	25.098444, 56.109218	Nashat Hamidan
<i>Garra barreimiae</i> Shawkah UAE	NH UAE-012	Ex91F12	25.098444, 56.109218	Nashat Hamidan
<i>Garra barreimiae</i> Shawkah UAE	NH UAE-013	Ex91G1	25.098444, 56.109218	Nashat Hamidan
<i>Garra barreimiae</i> Shawkah UAE	NH UAE-014	Ex91G2	25.098444, 56.109218	Nashat Hamidan

DNA extraction and PCR

Mitochondrial DNA was extracted from the fish tissues using Macherey and Nagel NucleoSpin® Tissue kits following the manufacturer's protocol on an EppendorfEpMotion® pipetting-roboter with vacuum manifold.

The standard vertebrate DNA barcode region of COI (cytochrome c oxidase subunit 1) was amplified using a M13 tailed primer cocktail including FishF2_t1 (5' TGTAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC), FishR2_t1 (5' CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA), VF2_t1 (5' TGTAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC) and FR1d_t1 (5' CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCAR-AA) (Ivanova et al., 2007). Sequencing of the ExoSAP-IT (USB) purified PCR product in both directions was conducted at Macrogen Europe Laboratories with forward sequencing primer M13F (5' GTAAACGACGGCCAGT) and reverse sequencing primer M13R-pUC (5' CAGGAAACAGCTATGAC). In order to better understand the phylogenetic position of the studied species, records were included from the NCBI Genbank for *Garra congoensis* (DRC), *G. ornata* (DRC), and *G. waterloti* from Guinea. Kimura 2-parameter

(K2P) model was used to measure the distance since it is the simplest, most symmetrical model that has different rates for transitions than for transversions (Kimura, 1980).

Molecular data analysis

To determine the most appropriate sequence evolution model for the data and thus enable hypothesis testing, the molecular data processing and sequence assembly was done in 'Geneious' (Biomatters, 2013), with the Muscle algorithm (Edgar, 2004) chosen to create a DNA sequence alignment. The most appropriate sequence evolution model for the given data was determined using the program 'Modeltest' (Posada and Crandall, 1998), implemented in the MEGA 5 software (Tamura et al., 2011), treating gaps and missing data with the partial deletion option under 95 % site coverage cut-off. The model with the lowest BIC score (Bayesian Information Criterion) was used to best describe the substitution pattern. Neighbour-joining (Saitou and Nei, 1987), maximum parsimony (Swofford, 2002; with PAUP4b) and maximum likelihood phylogenetic trees were generated with 1000 bootstrap replicates to explore species phylogenetic affinities, and thus test the hypotheses.

3.3 Results

The Maximum Likelihood based estimation of the phylogenetic relationships, based on the mitochondrial COI barcode region, placed the sequenced fishes into 24 groups (Figure 13). This revealed values in the K2P sequence divergence in their COI barcode region of between 0.2 (for *Garra congoensis* vs. *Garra ornate*, indicating a close phylogenetic relationship) and 36.2 % (for *G. ornata* vs. *G. rufa*, indicating a relatively distant phylogenetic relationship). It also revealed that *G. ghorensis* is distantly related to the South Arabian *Garra* species of *G. tibanica*, *Garra buettikeri*, *Garra dunsirei* and *Garra*

smartii with a minimum K2P distance of 15.6 % to this group (Figure 13) while closer to the Mesopotamian/ Mediterranean group of *G. rufa*.

The *G. rufa* from the Euphrates and Tigris Rivers formed a group that was well separated from the fish identified as *G. rufa* from the Orontes to the Jordan drainages. The *Garra* populations of the Jordan drainage were closely related to *G. ghorensis* in the southern Dead Sea and so could not be identified as *G. rufa*, as suggested by Krupp and Schneider (1989). Instead, the phylogeographic outputs suggested they are a new species, i.e. *Garra jordanica*, as per Hamidan et al. (2014) (Figure 12, 13). The phylogenetic tree is also assured the existence of the undecided species of *Garra* of the Orontes.

3.4 Discussion

Section 3.2 developed three hypotheses in relation to the origin of the *Garra* genus. H1 and H2 related to the biogeographic origin of *G. ghorensis*, either the Arabian Peninsula (H1) or the Mediterranean and Mesopotamia (H2, and H3 related to the potential presence of a *Garra* species yet to be described (potentially *Garra jordanica*). These are now discussed in turn. The phylogenetic tree built from the mitochondrial DNA sequences rejected H1 that had the origin of *G. ghorensis* as the Arabian Peninsula, due to their morphometric similarity to *G. tibanicus*. Instead, the output was consistent with H2, that *G. ghorensis* shared a common ancestor with *G. rufa*, with their biogeographic origin being the basins of the Mediterranean and Persian/Arabian Gulf basins, with close phylogenetic relations with other species such as *G. barreimiae*, *G. widdowsoni* and *G. jordanica* (Goren and Ortal 1999; Gorshkova et al., 2012). Krupp (1982) had described *G. ghorensis* as a subspecies of *G. tibanicus* due to their high morphological similarity of many *Garra* species in Western Asia, but this was clearly refuted genetically.

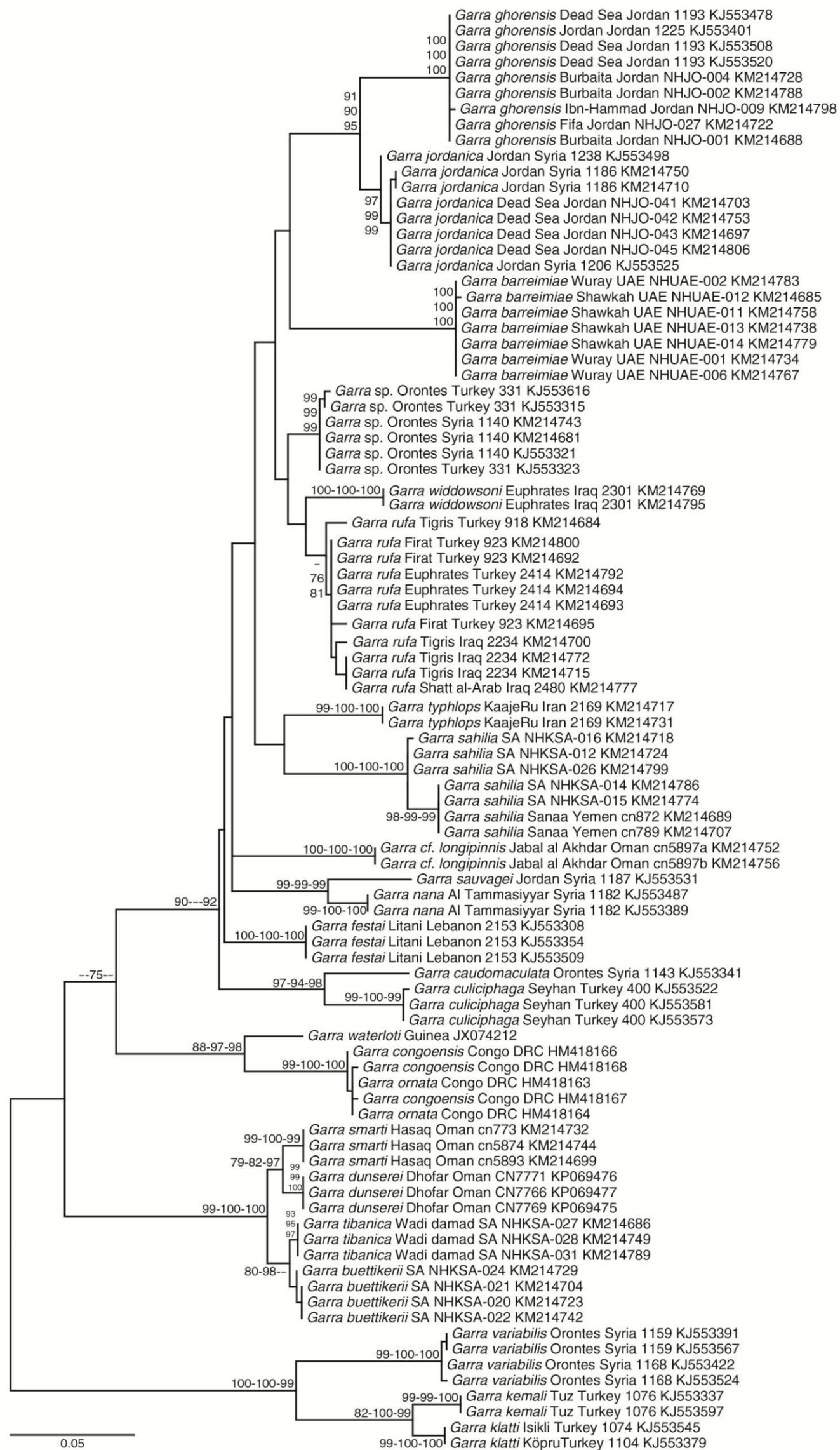


Figure 13 Maximum Likelihood estimation of the phylogenetic relationships of 24 *Garra* species from the Arabian Peninsula, the Mediterranean basin, Mesopotamia and Africa based on the mitochondrial COI barcode region. Nucleotide positions with less than 95 % site coverage were eliminated before analysis. Numbers of nodes indicate bootstrap values (> 75 %) from the Maximum Likelihood, Neighbour joining, and Maximum Parsimony method based on 1000 pseudo replicates.

Krupp (1985) and Krupp and Schneider (1989) have indicated that there is a high proportion of endemic freshwater fishes in the Dead Sea and Jordan basin, and postulated that the Jordan River had been directly, or through the Damascus basin, been colonised by fishes from the Euphrates. It is now apparent by the phylogenetic analysis that the ancestral population of *G. ghorensis* and *G. jordanica* colonised the Dead Sea basin from the Euphrates. As this connection was already closed during the Pliocene (Horowitz 1979), then this was in broad agreement to the phylogenetic tree here that indicated *G. ghorensis* and *G. jordanica* were only distantly related to *G. rufa* from the Euphrates.

The presence and/or absence of *G. ghorensis* in the southern Dead Sea basin might be explained by the variable salinities of both Samra and then Lisan lakes (Section 2.2). At that time, the common ancestor of *G. jordanica* and *G. ghorensis* inhabited the coast of Samra Lake, following the north-south pathway through the wadis on both sides of the lake. Samra Lake was succeeded by Lisan Lake, with this extending from the present Lake Tiberias in the north to Wadi Khneizereh in the south of the Dead Sea. The high salinity of Lisan Lake (Abed, 1985) disconnected the *G. jordanica* populations at the Mujib River. *Garra ghorensis* did appear to survive in this period at Wadi Ibn-Hammad due to its high freshwater flow, although it was disconnected between the Mujib River and Wadi Ibn-Hammad. It is then proposed that the southern population remained connected under they were extirpated due to the salinity of Lake Lisan increasing to lethal levels. Thus, the fish species of this area that thrived in the freshwaters of Samra Lake were lost due to Lake Lisan, with this supported in contemporary times by the absence of any fish species in the wadis of this area (*cf.* Chapter 4).

Hypothesis 3 suggested that *Garra* fishes from the northern Dead Sea basin represented a different lineage than *G. rufa* from Mesopotamia, and thus are a species yet to be described (for simplicity, they have been referred to as *G. jordanica* throughout the

chapter). The analyses completed here support this. Krupp (1985) and Krupp and Schneider (1989) already pointed to the high rate of endemic freshwater fishes in the Dead Sea and Jordan basin, and postulated that the Jordan had been colonised directly, or through the Damascus basin, by fishes from the Euphrates. There is now little doubt that the population ancestral to *G. ghorensis* and *G. jordanica* invaded the Dead Sea basin from the Euphrates. This connection was already closed during the Pliocene (Horowitz, 1979), which is in good agreement with these findings since these fishes are only distantly related to *G. rufa* from the Euphrates. Krupp (1985) also pointed out that fishes might have reached the Orontes from the Jordan through the Litani, which flows in Lebanon, as the upper Litani has lost one of its headwater streams to the Orontes (Vaumas, 1957). Krupp (1985) gave one record of *G. rufa* for the Litani, although this is likely to be erroneous given that the species has not been found in this river despite intensive fieldwork in recent years (M. Bariche, personal communication). Also, the molecular data do not suggest an invasion of *G. jordanica* to the Orontes. Most likely, the Orontes was colonised by *Garra* fishes directly from the Euphrates at a later stage than the colonization of the Jordan from the Euphrates.

Thus, these analyses of *Garra* mitochondrial DNA has enabled testing of the three hypotheses. They revealed that *G. ghorensis* is (genetically) closely related to *Garra* species from the Mediterranean and Persian/Arabian Gulf basins, and this helps to explain their biogeography, ancestral populations and their dispersal throughout the distribution range. They have also indicated that *G. jordanica* can also be described as a new *Garra* species in their own right.

Chapter 4. A revised account of the geographical distribution of the endangered freshwater fish *Garra ghorensis* in Jordan and implications for conservation

A version of this chapter has been published as:

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4.1 Introduction

The causal factors involved in extirpations and extinctions of threatened freshwater fishes include the negative consequences that arise from anthropogenic disturbances including engineering works, industrial and domestic pollution, acidification, fishing and fishery management, and land use practices (Maitland, 1995; Olden et al., 2010; Maceda-Veiga, 2013). Consequently, the successful conservation of freshwater fish is highly reliant on data on their ecology and distribution, and understanding their relationships with their physical habitats (Dudgeon, 2000). Indeed, understanding how species respond to disturbances is important for understanding how human activities affect key habitats, such as spawning and nursery areas (Maitland, 1995; Olden et al., 2010). Maintaining habitat connectivity is especially important for species that undertake spawning migrations, with impoundments usually resulting in losses of both longitudinal and lateral connectivity (Falke and Gido, 2006; Fullerton et al., 2010). Data on the consequences of habitat alterations on threatened fishes are, however, often either unavailable or expensive to collect, especially in remote areas and where countries have limited conservation resources (Helfmann, 2007). This can result in conservation efforts often being undermined by insufficient understandings on the ecology and distribution of the species.

The importance of understanding the distribution and ecology of threatened freshwater fishes is highlighted by the genus *Garra* of the Cyprinidae family that has attracted attention and dispute in their taxonomic and biogeographic origins (Hamidan et al., 2014; Chapter 3). This genus is encountered across subtropical and tropical Asia, the Middle East and Africa (Menon, 1964), with ten species recognised by Geiger et al. (2014) in the Mediterranean basin. Of these ten species, four have a mental adhesive disc, being *G. variabilis*, distributed in the Asi and Nahr al Kabir drainages in Syria, *G.*

ghorensis, distributed in the southern tributaries of the Dead Sea basin, but currently only found in Jordan (Hamidan and Mir, 2003), *G. jordanica*, distributed in the northern Dead Sea basin of Jordan and Syria, and *G. rufa*, distributed in the Qweik, Euphrates, Tigris and in rivers in the Persian Gulf south to the Mond River (Hamidan et al., 2014). *Garra ghorensis* was originally described by Krupp (1982) as a subspecies of *G. tibanica*, an Arabian species closely related to, or even identical with the African *G. quadrimaculata* (Stiassny and Getahun, 2007). However, the recent genetic study indicated that *G. ghorensis* is of Mediterranean and Mesopotamian origin (Hamidan et al., 2014; Chapter 3).

The distribution of *Garra* fishes in Jordan was discussed further by Krupp and Schneider (1989) and Mir (1990). These studies provided a comprehensive account of the fish fauna of Jordan and adjacent areas. These data were used as the basis of a review of the conservation status of freshwater fishes in the Arabian Peninsula, including southern and eastern Jordan, at a conservation assessment and management planning (CAMP) meeting in 2002. The outcome was a conservation plan outlining that three Jordanian fish species, *Aphanius ricardsoni*, *A. sirhani*, and *G. ghorensis*, were priority species for conservation as they faced an imminent risk of extinction (EPPA, 2002). At that time, *G. ghorensis* and *A. sirhani* were evaluated on the IUCN Red List as a critically endangered species while *A. ricardsoni* as endangered. A recent evaluation has reduced *G. ghorensis* to endangered status due to their area of occupancy not allowing for the classification of critically endangered (Freyhof, 2014).

Despite this conservation prioritisation in 2002, there was a paucity of data on the status and distribution of these fishes, including *G. ghorensis*. This presented a major challenge to any efforts to conserve these species in light of potential impacts of anthropogenic disturbances (e.g. impoundments) and climatic events (e.g. drought). Correspondingly, Hamidan and Mir (2003) assessed the status of *G. ghorensis* in Jordan

in 2002, building on knowledge provided by earlier studies of Krupp and Schneider (1989) and Mir (1990). Since this survey, however, there have been substantial alterations to many natural watercourses in Jordan, such as the construction of impoundments that have transformed lotic habitats to lentic in order to meet societal demands for potable water and irrigation.

One of the large-scale water impoundments in the distribution range of *G. ghorensis* (Figure 4) was Al-Tannour Dam that receives water of a catchment area of 2160 km sq, and located on the Wadi Al-Hassa system (Hadadin, 2015; Figure 2), which is one of the most important wadi system of *G. ghorensis* (Hamidan and Mir, 2003). In addition to the Al-Tannour Dam, four local impoundments with 30,000-100,000 cubic metre capacities were established downstream to regulate water use for agriculture and these were on Ein Al- Haditha (HD) site, Wadi Al-Hassa, Wadi Fifa, and Wadi Khneizerah (Figure 4).

This shift in lotic characteristics, allied with reduced volumes due to water abstraction and the introduction of alien species (e.g. *Oreochromis aureus*), suggest there has been some anthropogenic disturbances that could potentially have impacted the status of populations of *G. ghorensis* since the 2002 surveys (Hamidan and Mir, 2003). Consequently, the aims of this chapter were to (1) assess the spatial distribution of *G. ghorensis* in Jordan in 2010 and compare it to the distribution recorded by Hamidan and Mir (2003); (2) assess the extent of the increased anthropogenic disturbances at the survey sites in 2010 compared with 2002, and (3) identify the issues that could result in conservation threats to the current status of *G. ghorensis*.

4.2 Materials and Methods

4.2.1 Study area

Sampling for *G. ghorensis* in 2010 was conducted in October at 14 riverine sites at the southern end of the Dead Sea in Jordan. Of these sites, six had been sampled in 2002, with a further eight sampled here to identify other sites where the species might be present (Table 3; Figure 4). Of the six sites sampled in both years, four were impounded in their lower reaches where the water used to drain to the Dead Sea.

In entirety, the spatial area covered in the 2010 surveys encompassed the distribution range of *G. ghorensis* as reported by Krupp (1982), Krupp and Schneider (1989), Mir (1990), Hamidan and Mir (2003) and Hamidan (2004). It is thus comprised the area from Ein Al-Haditha (31°17'47.74" N, 35°32'35.38"E) at the northern border and extended south to Wadi Khneizerah (30°52'53.79"N, 35°26'1.00"E) app. 50 km to the south of Ibn Hammad. It also extended east to Wadi al-Burbaitah (30°59'1.11"N, 35°40'13.71"E) at the upper tributaries of Wadi Al-Hassa (31° 0'44.95" N, 35°31'19.08"E), and from western site to rivers outlets down to the Dead Sea (Figure 14). A brief description of each site is provided in Table 3.

4.2.2 Fish sampling

Fish sampling at the 14 sites was completed in October 2010. At all sites, sampling used electric fishing. Where sites were impounded, then the downstream limit of the site would be the impoundment. Sampling was completed at all sites in an upstream direction and continued for 15 minutes before moving 500 m upstream to repeat. This was repeated once more so that a total of 45 minutes fishing was completed per site and over a distance of approximately 1500 m river length. The electric fishing equipment was a hand-held Samus 725 MP electro-fishing unit. At each section of each site,

sampling concluded before 15 minutes if 15 *G. ghorensis* individuals were captured. This was to prevent excessive numbers of this endangered fish being captured.

With the exception the sites Ain al-Hadihta, Wadi Ibn-Hammad, and Wadi Burbaitah, field identification of *G. ghorensis* was completed at the conclusion of the fishing and then all fish were immediately returned to the water to prevent excessive handling and stress associated with capture. As sample sizes at Ain al-Hadihta, Wadi Ibn-Hammad and Wadi Burbaitah were relatively high, then up to 15 individual fish were removed, euthanized (overdose of anaesthetic, clove oil) and taken back to the laboratory for identification and measuring (standard length, nearest 0.1 mm). Permission for removing individuals was granted by licence from the Royal Society for the Conservation of Nature, Jordan.

Across the 14 sites, reporting of *G. ghorensis* was on a presence/ absence basis, with supplementary data only provided for Ain al-Hadihta, Wadi Ibn-Hammad, and Wadi Burbaitah. Qualitative assessment of the extent of anthropogenic disturbance was through noting the additional alterations to the sites since 2002. As length data were available in both 2002 and 2010 for the site at Ein Al-Haditha, differences in the length distribution of *G. ghorensis* between the years were tested using a Mann Whitney U-test, as they were not normally distributed (Shapiro-Wilk test, $P < 0.05$ in both years). In reporting, where error is expressed around the mean, it represents standard error.

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Table 3 The sites sampled in 2010 across the described range of *G. ghorensis*; presented from north (Ibn-Hammad) to south (Wadi Khneizereh). In site name, codes in parentheses refer to sites used in Table 2. Year represents the year(s) the sites were sampled

Site name	Location	Alt.*	Year	Brief description	Human impact		Fish species
					2002	2010	
Ibn-Hammad (IB)	31°18'4.25" N, 35°37'47.36" E	81	2002, 2010	Deep cliff, shallow water (10-30 cm), fast running (app. 1.2 m/s). Width of sampling site: 2-4 metres, and depth is less than 10 m.	Minimum number of visitors with no facilities. Water extraction for agriculture at a local scale.	Over visit especially in summer, tourism facilities Agricultural encroachment on the wadi beds, and water extraction	<i>G. ghorensis</i>
Ain al-Haditha (HD)	31°17'47.74" N, 35°32'35.38"E	-316	2002, 2010	Local impoundments, natural water pond at the spring head (app. 28m Length by 4-8 m width), deep (3 m), artificial concrete collection ponds, and a fast running (1.3 m/s) open channelled water between the natural and artificial ponds.	Impoundment Water extraction for agriculture, recreation, and invasion with <i>O. aureus</i>	Large scale water extraction to apply the growing demand of agriculture, invasion with <i>O. aureus</i> , grazing around the natural ponds and livestock drinking, and recreation.	<i>G. ghorensis</i> <i>Oreochromis aureus</i>

Site name	Location	Alt.*	Year	Brief description	Human impact		Fish species
					2002	2010	
Wadi al-Hassa	31° 0'44.95" N, 35°31'19.08"E	-184	2002-2010	Drainage system for several tributaries and springs extending along the Karak Mountains	Tourism activities, water extraction,	Large scale dam up stream, Tourism facilities, Over visiting at both Afra and Burbaita site. Enlarged agricultural scheme, water extraction, and river diversion especially at the upper reaches.	<i>G. ghorensis</i> <i>Capoeta damascina</i>
Afra hot spring	35°38'24.96"E 30°59'2.97"N	180	2002-2010	Originated from Wadi al-, sulphuric hot spring with temperature of almost 40c° originated from the main Afra hot spring 2.6 km from the confluence point with Wadi al Burbaitah. Fast running wadi (1.3 m/s), with long gorge, narrow 1 m width to wide edges 20 m width especiall at the lower part.			<i>G. ghorensis</i> (Only juvenile fish were found close to the confluence point with wadi Burbaitah)
Wadi-al-Burbaita (BR)	30°59'1.11"N, 35°40'13.71"E	250	2002-2010	Originated from Wadi al-Hassa at the confluence point with Afra hot			<i>G. ghorensis</i> <i>C. damascina</i>

Site name	Location	Alt.*	Year	Brief description	Human impact		Fish species
					2002	2010	
				spring. Fast running water			
Wadi Fifa	30°55'52.57"N , 35°28'46.55"E	-260	2002- 2010	Fast running (1.4m /s) narrow width 1-3m wadi.	Domestic use of water	Water impoundment, large scale water extraction to apply the expanded agricultural demand on water, recreation activities including over visiting, grazing and livestock drinking.	<i>G. ghorensis</i> <i>C. damascina</i>
Wadi Khneizereh	30°52'53.79"N , 35°26'1.00"E	-256	2002- 2010	A narrow wadi surrounded by hills of sandstone and limestone with large boulders, Fast running water (1.3 m/s) water depth varies from 10 -50 cm depth)	Domestic use of water Water extraction for agriculture. Impoundment at the downstream. Tourism.	Increased water extraction to apply the expanded agricultural demand. Over visiting / recreation.	<i>G. ghorensis</i> <i>C. damascina</i>
wadi al-Karak	31°15'32.11" N, 35°36'50.68"	-51	2010	Fast running river (0.9 m/s) water depth is 15-30 cm, wadi width is varied from 1 m water width to 12 m	Water extraction for agriculture	Increased water demand, that block the water to reach the downstream leaving behind a dry wadi of 1.5 km river length, the wadi became over visited by tourists at local and	<i>Oxyneomacheilus insignis</i>

Site name	Location	Alt.*	Year	Brief description	Human impact		Fish species
					2002	2010	
				at the eastern side.		national levels.	
Wadi Weida'a	[31°13'45.29" N, 35°34'51.67"E	50	2010	perennial shallow and slow running stream (0.3 m/s)	Tourism activities	Tourism activities	-
Wadi 'Assal	31°11'16.40"N , 35°33'39.98"E	-190	2010	perennial shallow and disconnecting water flow.	Tourism activities	Tourism activities and grazing around the site	-
Wadi Marsad	31°10'24.81"N , 35°32'38.02"E	-250	2010	little, shallow, and slow running (0.3-0.1 m/s) streams of water that is not extended over the wadis	Tourism activities	Tourism activities and grazing around the site.	-
Wadi Numeira	31° 8'59.69"N, 35°32'9.91"E	-266	2010				
wadi Hudeira	31° 7'49.81"N, 35°32'1.73"E	-245	2010	Slow running (0.3m/s) and low amount water that is extended to a dead end	Tourism activities	Tourism activities, grazing around the site, and water collection in artificial ponds for domestic and agricultural use.	-
wadi Umruq	30°54'7.14"N, 35°28'51.69"E	-150		Thick and heavy riparian vegetation that is covering the little amount of	Water extraction for agriculture Local tourism	Large water extraction for agricultural purposes, High tourism activities at national level.	-

Site name	Location	Alt.*	Year	Brief description	Human impact		Fish species
					2002	2010	
				water along of the wadi			

* Refers to altitude at the collection site

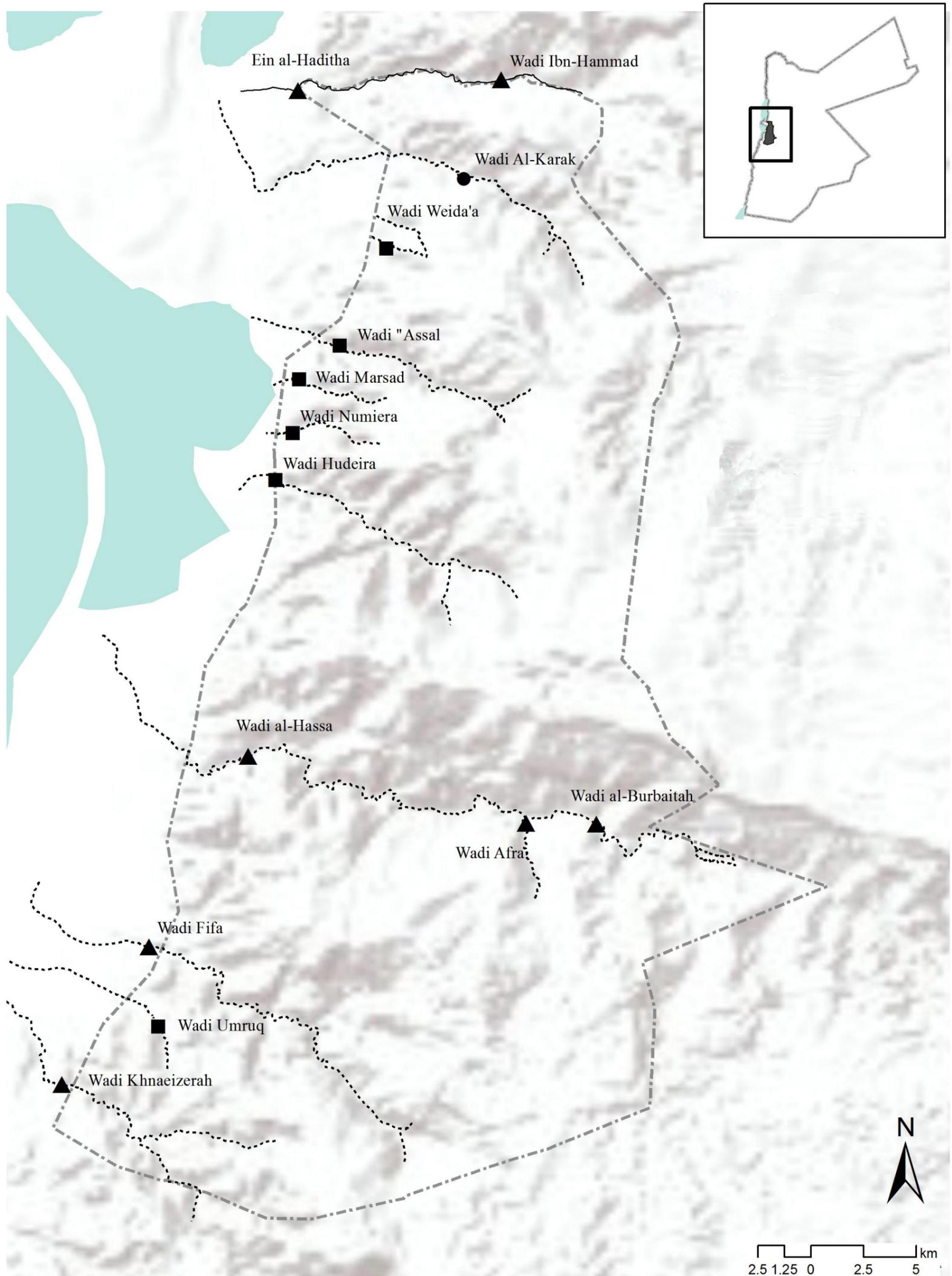


Figure 14 Locations of the sampling sites in Jordan (inset) and in Southern Jordan (main image). Filled triangles represent sites where *Garra ghorensis* was present, filled squares represent sites where they were absent. The filled circle is the site where only *Oxyneomacheilus insignis* was captured. The dashed line marks the limit of the known up to date distribution range of *G. ghorensis* described by Krupp and Schneider (1989), Mir (1990), Hamidan and Mir (2003), and Hamidan (2014).

4.3 Results

In 2002, *G. ghorensis* was detected in all of the six sites that were sampled (Figure 2) (Hamidan and Mir 2003). In 2010, all of these sites were still found to have populations, with an additional population detected in one of the eight extra sites fished (Table 3). This was an allopatric population in the lower reaches of Wadi Ibn Hammad (Table. 3). The samples collected in 2010 revealed that the seven recorded *G. ghorensis* populations comprised: (i) an isolated population at the lower part of wadi Ibn Hammad; (ii) a population that was sympatric with invasive *O. aureus* in Ein Al-Haditha; and (iii) populations that coexisted naturally with the native *Capoeta damascina* (Wadi Al-Hassa (including Burbaitah and wadi Fifa), and wadi Khneizerah) (Table 3). In the upper part of Wadi Al-Karak, no *G. ghorensis* were sampled, but the nemacheilid loach *Oxyneomacheilus insignis* was found (Figure 14, Table 3). There were no fish recorded from Wadi Weida'a, Wadi Assal, Wadi Marsad, Wadi Numeira, Wadi Hudeira, and Wadi Umruq (Figure 14, Table 3). Across the three sites where samples were recorded in more detail, the number of sampled *G. ghorensis* was the lowest at wadi Ibn Hammad (n = 9) and highest at Wadi al-Burbaitah (n = 78) (Table 4).

Table 4 Sample size, sub-sample size and length characteristics of *Garra ghorensis* at the three sites where they were most abundant. Site codes are those referred to in Table 3.1. Site Codes: Ain al-Haditha (HD), Ibn-Hammad (IB), and Wadi al Burbaitah (BR).

Year	Site code	Number fish sampled	Sub-sample size (n)	Mean length (mm)	Length range (mm)
2002	HD	123	-	57.4 ± 1.3	29.0 - 99.0
2010	HD	15	15	35.1 ± 2.6	20.7 – 48.8
2010	BR	78	15	45.2 ± 2.8	24.9 – 62.0
2010	IB	9	9	32.7 ± 3.4	21.2 – 57.3

Data of collected samples at Ein Al-Haditha in 2002, and the three sites in 2010, revealed *G. ghorensis* present in samples from 21 to 99 mm, suggesting a recruiting population comprising of juvenile and mature fish (*cf.* Chapter 5), with mean lengths highest in Ein Al-Haditha and lowest in Ibn Hammad (Table 4). Between the samples collected in 2002 and 2010 at Ein Al-Haditha, their median standard lengths differed (2002: 55.0 ± 1.2 mm; 2010: 35.1 ± 2.6 mm), with these differences being significant (Mann Whitney U test: $Z = -4.95$, $P < 0.01$).

Additional anthropogenic disturbances were apparent at all sites sampled (Table 3). The primary disturbances were increased impoundment, leading to decreased flows, channel deepening and the potential for increased silt deposition (Table 3). At a larger spatial scale, a 17 million cubic metres dam, Al-Tannour, was established at the upstream of Wadi Al-Hassa and was operational from 2005, after which seasonal flooding was

controlled and non-native fishes were introduced into the impoundment (*O. aureus*, *Cyprinus carpio*, and *Clarias gariepinus*). However, no introduced fish were present in the samples collected downstream.

4.4 Discussion

The 2010 surveys revealed that across the range of *G. ghorensis* described by Krupp (1982), Krupp and Schneider (1989), Mir (1990), Hamidan and Mir (2003) and Hamidan (2004), seven populations were detected, of which six had previously been detected in 2002. These populations covered three scenarios: allopatry, present in sympatry with native *C. damascina* and present in sympatry with invasive *O. aureus*. This reveals that *G. ghorensis* is present in fish communities with inherently low species diversity, perhaps due to the often extreme conditions that occur at the sites. These conditions include very high summer water temperatures (> 30 °C) and low flows, and winter flood events (Hamidan and Mir, 2003). The influence of these invasive species on the ecology of the populations of these sites is discussed further in Chapters 5 to 7.

In comparison with 2002, the six sites re-sampled in 2010 all revealed additional physical modifications from anthropogenic disturbances, particularly at the lower reaches close to their confluence to the Dead Sea, where the water tended to be impounded and/ or heavily abstracted for domestic and agricultural use. With the exception of the Al-Tannour dam, these schemes tended to be relatively small-scale. As these impoundments are mainly at the lower end of the rivers, then their potential impacts of *G. ghorensis* were likely to relate more to shifting conditions from lotic to lentic, rather than being connected to population fragmentation. Across the seven populations, although no apparent major issues were yet apparent for *G. ghorensis* from these impoundments, it should be noted that these surveys were restricted in scope, with a primary focus on the presence/ absence of the species and so restricting further

inferences on the effects of habitat change on other aspects of their ecology (*cf.* Chapters 5, 6).

The use of impoundments to manage freshwater availability in water-poor countries such as Jordan is only likely to increase in future, particularly as climate change predictions for the country are for increasing uncertainty in rainfall patterns, including increased periodicity of drought and thus water shortages (United Nation for Development Programme/ Ministry of Environment, 2014, Hadadin, 2015). Impoundments thus represent an increasing conservation threat to the sustainability of Jordanian freshwater resources and the fish communities they support. Although they might provide some benefits in minimising the harmful effects of annual flood cycles, and especially the damaging effects of stochastic summer flood events on fish recruitment, flooding can also play important ecological and engineering roles in river systems (Jackson, 1989, Kingsford, 2000; Olden et al., 2005). Moreover, impoundments tend to provide conditions suitable for the establishment of introduced fishes (Johnson et al., 2008; Liermann et al., 2012), a result of the considerable disturbance that occurs when lotic conditions are transformed to lentic that are unfavourable for native fluvial fishes allied to the introduction of non-native fishes for recreational angling and/ or aquaculture (Liermann et al., 2012). Indeed, non-native fishes such as *Tilapia zillii*, *O. aureus* and *C. carpio*, and *Clarias gariepinus* already been introduced into some impoundments in Jordan (Hamidan, 2014), primarily for fishery purposes (Khoury et al., 2012). Should these species develop invasive populations then there would be potential for detrimental ecological consequences to develop (Gozlan et al., 2010; Simberloff et al., 2013).

Impoundments also change the hydrological system by creating static water bodies (Lentic), which is not the favourable habitats for *G. ghorensis*, since the species prefers lotic habitat with fast running water, shallow with gravel bottom, described by Krupp

(1982). Effect of such habitat change from lotic to lentic is discussed later in this thesis in chapter 5.

In conclusion, despite a range of additional anthropogenic disturbances across their range, the distribution range of *G. ghorensis* did not decrease between 2002 and 2010, although the habitat changes are likely to have resulted in some ecological and life-history changes (Chapter 5, 6). These data provide some support for the recent downgrading of *G. ghorensis* from critically-endangered to endangered on the IUCN Red List, (Freyhof 2014), however, given the species' on-going and increasing disturbances from human activities, then it is suggested that their Red List status remains at endangered for the foreseeable future.

Chapter 5. Age structure and somatic growth rates of *G. ghorensis* in relation to varying levels of environmental disturbance

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5.1 Introduction

In arid regions, the escalating demands for water have resulted in the substantial modification of many river systems (Propst et al., 2008). In conjunction with the widespread invasion of many of these rivers by non-native fishes, this has increased the threat of local native fish populations being extirpated and endemic fishes becoming extinct (Kingsford, 2000; Olden and Poff, 2005; Propst et al., 2008). However, the risks of extirpation and extinction vary between species according to their traits that determine their responses to modified environments (McKinney, 1997; Olden et al., 2006, 2008). These ecological attributes, including their life history traits, ecological niche and morphology, have been applied to disturbed rivers in arid regions for identifying those species most at risk to extinction so that conservation strategies can focus on their populations (Olden et al., 2006, 2008; Pool and Olden, 2012). Studies suggest that fishes with a ‘slow’ life history, such as large body sizes, slow somatic growth rates and delayed maturity (i.e. K-selected traits), tend to have a greater frequency of local extirpation and are more prone to extinction compared with those with the opposite suite of traits (i.e. r-selected traits) (Olden et al., 2008).

In fish populations, life-span, age structure and somatic growth rates form an important component of their life history strategy through their relationships with reproductive traits such as length and age of maturity (Winemiller and Rose, 1992). Moreover, fish age and growth data assist the understanding of the basic ecological relationships of fish populations and their interactions with their environments (Beardsley and Britton, 2012; Britton et al., 2012). Given that life history traits can be a strong predictor of extirpations of fish populations in arid regions (Angermeier, 1995; Reynolds et al., 2005; Olden et al., 2006), the analysis of a species’ age composition and growth rates can help to explain how threatened species might be conserved in these regions by

indicating their initial responses to the environmental changes. Where data indicate, for example, significantly reduced life spans then this might suggest the species has relatively narrow tolerances to disturbance that ultimately could result in population decline and eventual loss.

In view of the endangered status of *G. ghorensis* outlined in Chapter 1, there is an impending requirement for their remaining populations to be conserved. However, there is little information available on the ecology of the species, with the only study (other than that detailed in this thesis) on their Jordanian populations being a distribution survey completed in the early 2000s (Hamidan and Mir, 2003; Chapter 3). As there has been no subsequent study that provides data on their life history traits then the aim here was to determine the age composition and growth rates of the three *G. ghorensis* populations identified in Chapter 3 and 4 as being appropriate for ecological studies and sampling. Through collecting monthly samples throughout 2011 and early 2012 (Chapter 4), the objectives were to:

- (1) identify the presence of length modes in the samples by month and assess their growth through 2011;
- (2) quantify the utility of using scales to age individual fish within the populations;
- (3) determine the age structure and growth rates of the three populations using scale analysis; and
- (4) identify the initial conservation implications for *G. ghorensis* of the outputs of Objectives 1 to 3.

5.2 Materials and Methods

Modal progression analysis (MPA) was used to identify the presence of length modes in the samples per month and assess their growth through 2011, enabling completion of Objective 1. Each month, the lengths of all the sampled fish at each site were sorted into length distributions (10 mm increments) and were then used within decomposition assessment using Bhattacharya's method in FiSAT (Bhattacharya, 1967; Bolland et al., 2007). This identified the presence of modes in each length distribution by separating them into a series of normal distributions (King, 2007). For each mode, the output was the number of individuals, their mean length and standard deviation (SD) (Bolland et al., 2007). The modes were separated by application of a separation index (SI), calculated as the ratio of the difference between successive means and the difference between their SD modes; values above 2.0 indicate significant difference from the other modes (Bhattacharya, 1967; Bolland et al., 2007). This is advantageous over similar methods as it ensures that the identified modes are significantly different and so is statistically reliable, justifying its selection for use over alternative methods (Bolland et al., 2007). The overall output of MPA for each site per month was the number of modes in the population and their mean length (\pm SD), enabling length increases to be identified for each mode over the study period.

Whilst MPA revealed the number of length modes in the population and their length increases over time, it could not reveal the age of the fish within the modes. Thus, to complete Objectives 2 and 3, the scales collected from the fish (Section 2.3) were analysed. To ascertain whether these fish could be aged from their scales (Objective 2), it was ascertained whether growth checks were present on scales. As they were then the next step was to determine the frequency and timing of their formation. This required the scales to be viewed on a projecting microscope (\times 48 magnification) and following

measurements taken from one scale: total scale radius (SR), distance from the focus to the last formed check (LA) and distance to the second-last formed check (LA-1). These data were then subjected to marginal increment ratio analysis (MIRA; Haas and Recksiek, 1995; Vilizzi and Walker, 1999), where the MIRA calculation of the marginal increment ratio (MIR) was determined by $MIR = [(SR - LA) / (LA - LA-1)]$. When only one check was observed, the denominator was the distance from the scale focus to the check (Vilizzi and Walker, 1999). To test for differences in the marginal increment ratio data for each month, ANOVA was used where the dependent variable was the MIR for each fish and the independent variable was month. Tukey's post-hoc tests enabled the significant differences to be identified for each month and indicated the timing of when the growth checks were formed.

Once the frequency of check formation had been determined then the age of each fish was determined by counting the number of growth checks. The scales were then measured to enable back-calculation of their lengths (Francis, 1990). This enabled three analyses on their lengths at age to be completed. First, the effect of sex on length at age was determined through building a general linear model (GLM) that tested the effect of sex (male or female; immature fish were excluded from the model) on length at the last annulus whilst the effects of site and age were controlled in the model. In the model, statistical complications from using repeated measurements from individual fish in the same test (i.e. pseudo-replication) were avoided by using only the back-calculated length at the last growth check for each fish (Beardsley and Britton, 2012). Differences between the sexes were assessed for significance using linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons. Second, the data were analysed for their mean standardised length-at-age residuals for each site (Benstead et al., 2007; Storm and Angilletta, 2007; Beardsley and Britton, 2012; Britton et al., 2012). This required the predicted mean length at each age across all the

populations to be determined using the log-log quadratic function of Vilizzi and Walker (1999) as this is the most precise and biologically meaningful growth model. These values then enabled the standardised residual of the length at age of each fish to be calculated (Beardsley and Britton, 2012), with these compared between sites using a GLM that controlled for the effects of sex. Again, only the back-calculated length at the last annulus was used for each fish to avoid pseudo-replication. Lastly, the length at age data by sex and site were applied to the non-linear von Bertalanffy growth model (von Bertalanffy, 1938) to produce values of the maximum (asymptote) theoretical length at each site (L_{∞}) and K , the annual growth rate towards L_{∞} . All statistics were completed in SPSS v.16.0 and only tests that met all underlying assumptions were used.

5.3 Results

5.3.1 Modal progression analysis

Across the study sites, a total of 123 *G. ghorensis* specimens were collected from HD site, total of 165 from IB site, and 164 from BR site (Table 5). Fish were captured to a maximum length of 137 mm, although the majority of fish sampled were below 100 mm (Figure 15). Modal progression analysis identified three length modes in each site, although not all modes could be identified every month (Figure 15). The smallest mode, whose mean lengths varied between 20 and 30 mm at IB and BR, but up to 48 mm in HD. They only appeared in samples from August at the earliest (Figure 15) in both BR and IB sites, but not in HD site until December, suggesting these fish were the fish that were hatched in the last spawning season technically known as young-of-the-year (YOY), and obviously were more abundant in IB and BR than in HD. At each site, there was also a length mode of relatively large fish (generally > 60 mm) whose growth increase was minimal through the year, especially at site HD (Figure 15) where they were high abundant than in BR, and IB respectively.

Table 5 Number and fork lengths of *Garra ghorensis* in sub-samples collected by site between February 2011 and January 2012. All lengths are in mm; where error is provided it represents standard deviation.

Month	HD			BR			IB		
	n	Mean length	Length range	N	Mean length	Length range	n	Mean length	Length range
February	15	76.0 ± 21.8	40.0 – 103.9	15	81.9 ± 22.9	43.1 – 113.4	0		
March	14	87.8 ± 12.6	68.6 – 105.2	15	50.3 ± 12.3	28.4 – 71.9	15	51.7 ± 11.2	35.1 – 73.2
April	15	74.7 ± 17.5	47.9 – 106.8	15	43.6 ± 12.9	23.8 – 60.8	15	53.1 ± 13.9	37.0 – 84.2
May	15	65.4 ± 18.4	33.1 – 88.9	15	59.1 ± 15.5	25.2 – 80.6	15	46.3 ± 17.2	20.9 – 70.9
June	15	77.4±17.5	42.0 – 113.4	15	44.8 ± 14.1	18.7 – 62.9	15	46.9 ± 14.4	25.0 – 75.4
July	4	65.8 ± 17.2	48.4 – 82.1	15	57.2 ± 15.3	34.5 – 88.6	15	53.0 ± 16.6	26.8 – 76.2
August	5	79.3 ± 27.5	54.5 – 126.7	14	43.3 ± 14.8	21.4 – 65.9	15	39.1 ± 11.2	24.6 – 55.1

Table 5 (cont.)		HD		BR			IB		
Month	n	Mean length	Length range	N	Mean length	Length range	n	Mean length	Length range
September	5	71.6 ± 15.7	94.5 - 56.8	15	44.0 ± 13.0	21.6 – 60.5	15	44.0 ± 15.9	21.6 – 60.5
October	4	51.5 ± 30.6	20.8 - 79.4	15	38.5 ± 14.7	15.7 – 69.4	15	49.0 ± 15.9	21.6 – 75.2
November	1	92.6		9	37.5 ± 10.9	25.7 – 63.1	15	49.6 ± 16.2	29.2 – 66.9
December	15	77.4 ± 14.4	45.4 - 101.9	6	26.8 ± 6.2	20.2 – 36.5	15	59.5 ± 16.2	26.1 – 74.2
January	15	69.8 ± 17.4	39.7 - 94.6	15	46.7±17.1	28.5 - 81.0	15	51.5 ± 13.1	31.3 - 65.7

5.3.2 Marginal increment ratio analysis of scales

There was a low number of fish sampled in the colder months and there were high volumes of regenerated scales in the samples generally. Note that these regenerated scales could not be aged as they had lost their original circuli patterns and so it was not possible to derive an age from them. This resulted in the number of scales (Figure 16) suitable for marginal increment ratio analysis and ageing being variable between months (range 0 to 15; see Table 5 for numbers per month and per site). The differences in the mean marginal increment ratios of scales in each site across the study period were significant (IB: $F_{10,76} = 6.61$, $P < 0.01$; BR: $F_{11,92} = 12.91$, $P < 0.01$; HD: $F_{9,88} = 36.21$, $P < 0.01$; Figure 17). At IB and HD, an increase in the marginal increments was apparent from April through to at least October, with post-hoc analyses (Tukeys) revealing significant differences between March and all subsequent months ($P < 0.05$; Figure 17).

At Site BR, growth at the scale margin was apparent from March, with post-hoc analyses (Tukeys) revealing significant differences between February and all subsequent months ($P < 0.05$; Figure 17). This suggests formation of an annual growth check in February/ March as the fish started to grow again after the colder winter period (Figure 15, 17).

5.3.3 Age range and structure

Scale ageing, completed by counting the number of annual growth checks (Figure 16), revealed fish present in the samples between 0+ and 6 years old (Figure 18). This indicated that the modes revealed by the MPA generally did represent discrete age-classes of fish (Figure 15, 17), where the smallest length-mode comprised young-of-the-year fish, the next mode comprised fish of age 1 in February/ March 2011 and 1+ thereafter, and the largest mode comprised of fish of > 2 years old (Figure 15, 17).

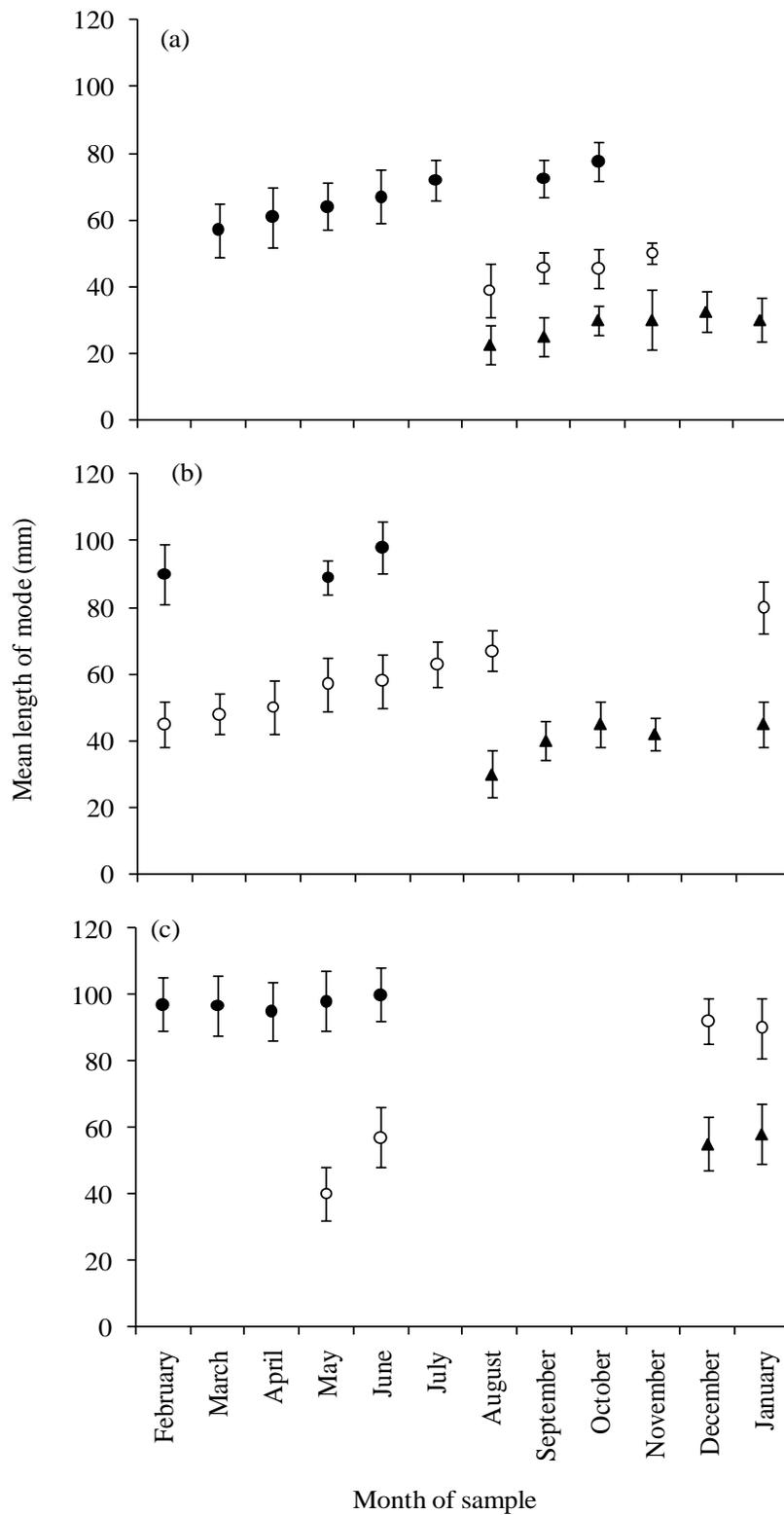


Figure 15 Mean lengths (\pm SD) per month of modes identified by modal progression analysis through the study period, where \blacktriangle = young-of-the-year; \circ age 1+; \bullet > age 2 years and (a) Site IB, (b) Site BR; and (c) Site HD.

The MPA could not differentiate different age-classes of fish within this largest length mode as the scales revealed their annual growth increments were relatively low compared to their growth earlier in life (Figure 15, 17). There was variation in the age structure between the sites, with only one fish aged above 3 years old at site IB, but with fish of age 4 and 5 present in greater numbers at Site BR and HD (Figure 18). There was only one 6-year-old fish present across all the samples (HD).

5.3.4 Length-at-age

Analysis of the age structure of the populations revealed a significant difference in the lengths at age of female and male fish across all sites ($F_{1,186} = 12.02$, $P < 0.01$; Figure 18), where both site and age had significant effects in the GLM ($P < 0.01$). Females were the larger sex, with an estimated marginal mean length of 62.7 ± 1.1 mm compared to 57.0 ± 1.2 mm for males; pairwise comparisons with Bonferroni adjustment for multiple comparisons indicated this difference was significant ($P < 0.01$). The lengths at each age of fish within each site were variable, with differences at age 1 by as much as 40 mm in fish whose lengths did not exceed 70 mm; Figure 18).

Length-at-age was also variable between the sites, with the mean standardized residual analysis revealing these differences were significant ($F_{2,185} = 19.19$, $P < 0.01$) when the significant effect of sex ($P < 0.01$) was controlled. Pairwise comparisons with Bonferroni adjustment for multiple comparisons indicated the significant differences were between site HD and both IB and BR ($P < 0.01$), but with no significant difference between IB and BR ($P > 0.05$; Figure 19). Outputs of the von Bertalanffy growth model also revealed that L_{∞} was highest at site HD, but relatively low at the other sites and with females having higher values than males (Table 6).

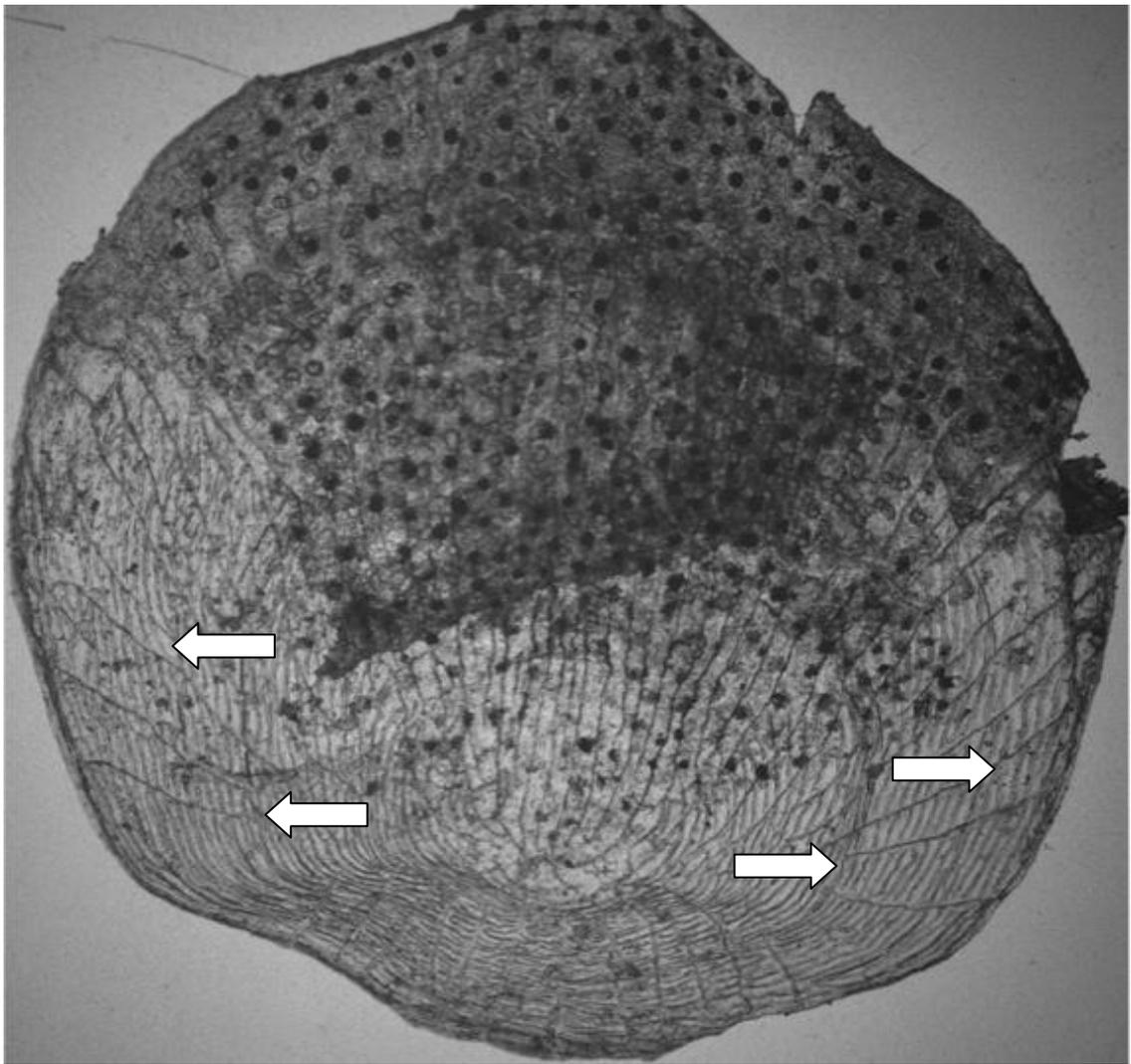


Figure 16 Image of a scale from a 67.3 mm *Garra ghorensis* sampled in May 2011 and age 2+ years. The white arrows mark the annual growth checks. Note the second growth check is close to the edge of the scale as a result of the time of sampling, i.e. the fish has only just started to grow again after winter.

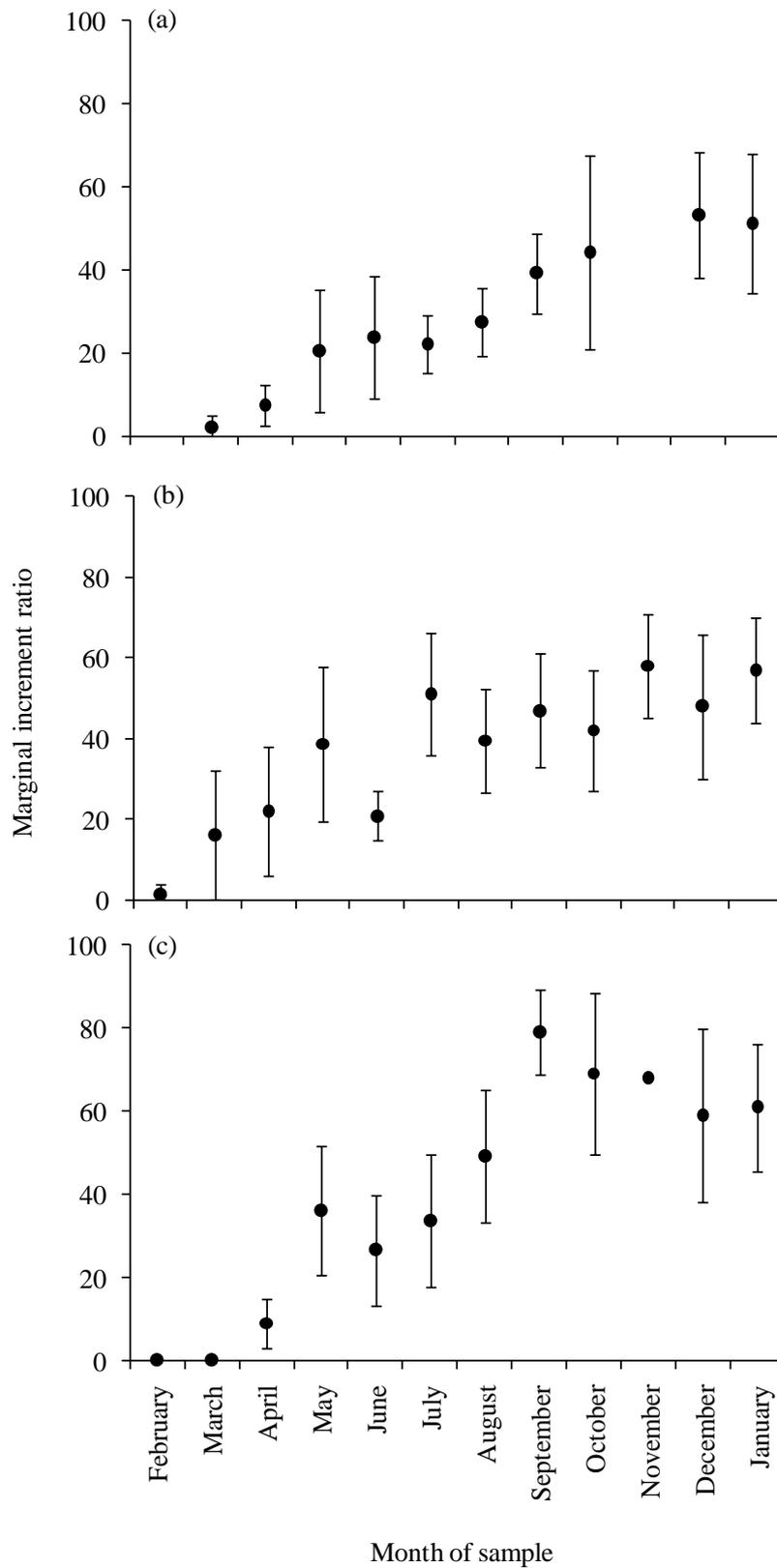


Figure 17 Marginal increment ratio analysis of scales (\pm SD) across the study period from (a) Site IB, (b) Site BR; and (c) Site HD.

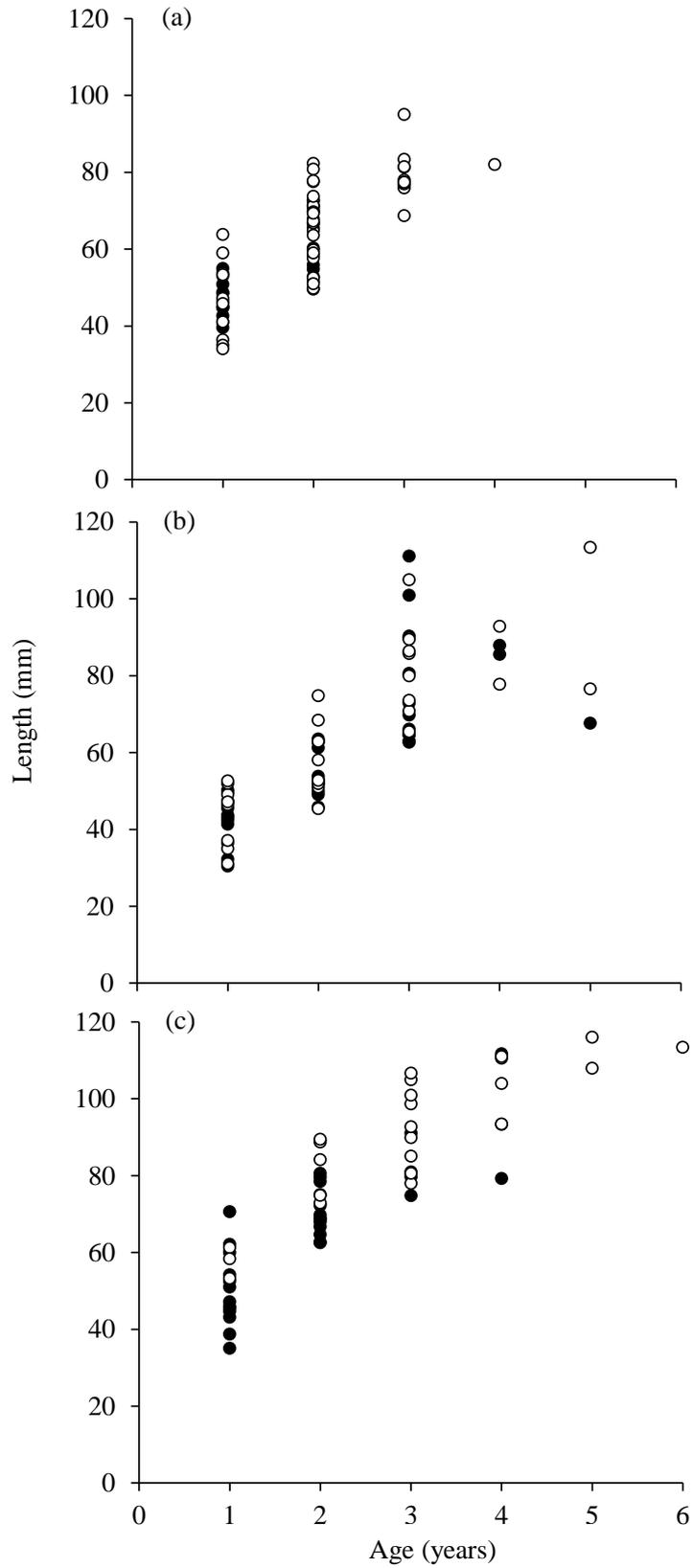


Figure 18 Length at the last annulus of ○ female and ● male fish at (a) Site IB, (b) Site BR; and (c) Site HD.

Table 6 Estimated parameters of the von Bertalanffy growth model for *Garra ghorensis* at the three study sites. Note at Site IB, values for male fish could not be calculated as fish were only present to age 2 years.

Site	Female		Male	
	L_{∞} (mm)	K	L_{∞} (mm)	K
IB	102 ± 8	0.36 ± 0.06	-	-
BR	112 ± 5	0.35 ± 0.04	80 ± 1	0.76 ± 0.07
HD	121 ± 2	0.48 ± 0.03	109 ± 1	0.47 ± 0.05

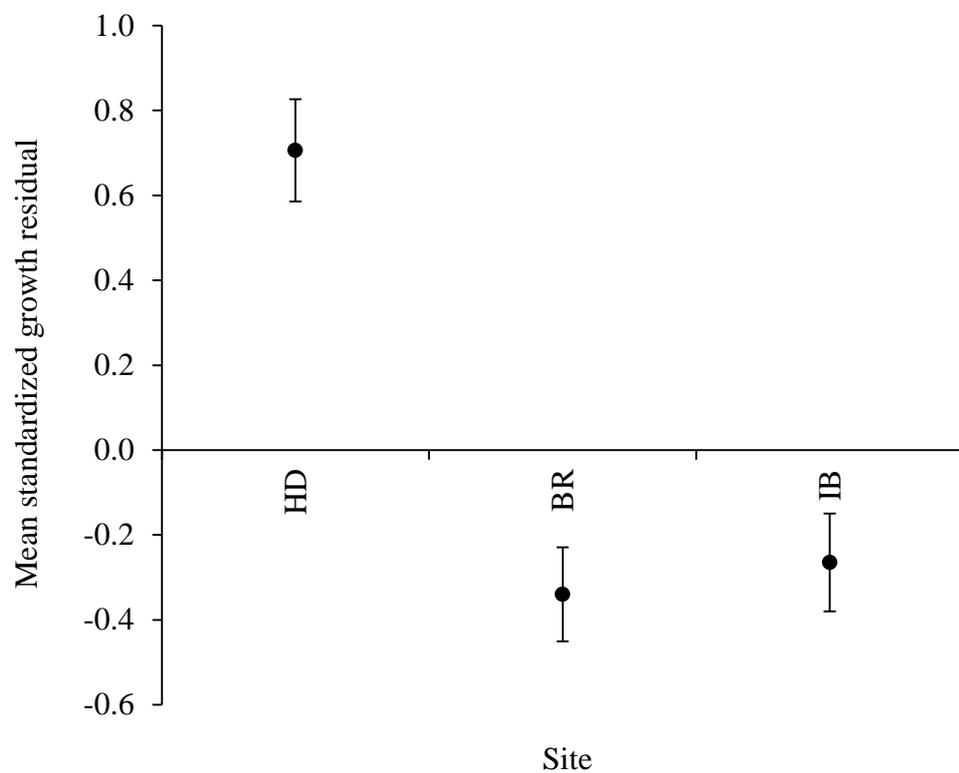


Figure 19 Estimated marginal means (\pm standard error) of standardized growth residuals at site HD, BR and IB, where the means have been adjusted for the effects of sex.

5.4 Discussion

The ages of *G. ghorensis* could be estimated from their scales and so provided data on the age structure, lengths at age and growth parameters of the three Jordanian populations. These data revealed individuals present in the sites of up to six years old, with relatively fast growth up to the age of two years and the production of relatively small annual growth increments thereafter. The oldest fish and fastest growth rates were recorded at site HD, the most disturbed site due to its habitat modifications including impoundment, substrate modification, and the presence of invasive *O. aureus* that became expanded to be 1:1 ratio with *G. ghorensis* (Hamidan, filed observation). Thus, the altered hydrological regime that increased depths and reduced flows were insufficient to impact the persistence of *G. ghorensis* and instead appeared to provide more optimal growth conditions than the less disturbed sites.

In disturbed arid environments, the life history traits of desert fish that increase their vulnerability to extirpation and extinction relate to those of the periodic life history strategy of Winemiller and Rose (1992) (Olden et al., 2006, 2008). This combination of large body size, late maturation, and low juvenile survivorship despite high fecundity per spawning event, results in poor adaptation to changing environments. By contrast, the traits of small body size, fast growth to maturation and low fecundity per spawning event are generally well predisposed to ensuring more favourable population responses to highly disturbed and unpredictable environments (Olden et al., 2006). These life history traits are important to understand given that desert fish conservation management strategies should be based on a fundamental understanding of how the ecological attributes of species interact with fluvial habitats to influence population persistence (Olden et al., 2008). In the case of *G. ghorensis*, their traits of relatively fast growth and limited lifespan suggest a life history strategy that is more aligned to the

opportunistic strategy of Winemiller and Rose (1992). They do not suggest the populations have 'slow' life histories that are congruent with high extinction risk (Olden et al., 2008), although it is acknowledged that data on *G. ghorensis* reproductive traits are required for this inference to be more robust.

This apparent resilience to disturbance in *G. ghorensis* was also emphasised by the population comprising of the longest-lived and fastest growing individuals being present in the most disturbed site. This is a key outcome given that many studies on threatened desert fishes in arid environments suggest that conservation strategies should focus on the restoration or maintenance of natural flow regimes (Poff et al., 1997; Eby et al., 2003; Richter et al., 2003). This is based on the assumption that the restoration of natural flow regimes will provide the impacted rivers with the attributes in which the native fauna evolved and so are necessary for the maintenance of robust and healthy populations (Propst et al., 2008). The data shown here for *G. ghorensis* suggested their populations can at least tolerate some hydrological disturbance - and also the presence of an invasive cichlid - suggests that their conservation management does not necessarily have to return their habitats to pristine conditions. Instead, the next steps in their conservation should be the identification of those life history traits (e.g. reproductive traits) and/ or ecological associations that do detrimentally impact their population persistence when their environments are disturbed so that these can be mitigated or rehabilitated (Olden et al., 2008). Notwithstanding, it should be noted that the impact of hydrological change is not necessarily clarified by the change in growth, but alternatively could be a result of displacement by juvenile fishes that reduces their number and thus results in reduced competitive interactions and so faster growth rates. Consequently, growth rate analysis alone is insufficient for drawing conclusions on the underlying effect of habitat alteration on *G. ghorensis* and hence why other metrics, such as diet composition, will also be assessed (*cf.* chapter 7).

There was high variability in the length at age of the fish at each site that was independent of sex, with variation between individuals at lengths of age 1 of up to 40 mm. This was also allied with the regular appearance of new 0+ fish in samples between July and October that were identified in a discrete length mode from August. Whilst it was not implicitly tested within the study, this suggests that a further favourable trait that provides *G. ghorensis* with some resilience to environmental disturbances is a protracted spawning period. This is because protracted spawning tends to produce high variation in the lengths of the 0-group cohort (Nunn et al., 2002). Protracted spawning periods are a common feature of many cyprinid fishes with, for example, *C. carpio* capable of asynchronous spawning throughout the year in equatorial regions (Britton et al., 2007). For other riverine cyprinid fishes, protracted spawning is also apparent. For example, in Lake Naivasha and its tributary rivers in Kenya, the cyprinid *Barbus paludinosus* spawns asynchronously with no clear spawning peak (Mutia et al., 2010). This is also the case in temperate cyprinid riverine populations, where protracted spawning periods provide the cohorts of juvenile fish with considerable resilience against stochastic environmental events that generally result in high early life mortality (Nunn et al., 2002). Here, the apparent protracted spawning by *G. ghorensis* might be a deliberate strategy that provides their progeny with the opportunity for greater proportions to survive their first year of life in systems that are located in arid regions of high summer temperatures and very low rainfall that could lead to periodic drying of some nursery areas and so high mortality of the 0+ cohort.

Chapter 6. Reproductive traits of *Garra ghorensis* in relation to varying levels of environmental disturbance

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6.1 Introduction

Life history traits are strong predictors of the vulnerability of fish populations to extirpation as they determine how resilient a species is to disturbances such as habitat loss and the adverse effects of invasive species (e.g., Angermeier, 1995; Reynolds et al., 2005; Olden et al., 2006, 2008; Dawson et al., 2011; Mace, 2014). Consequently, knowledge of life history traits provides a suite of information to inform the conservation of threatened fish species (Olden et al., 2006). The traits of reproductive effort, duration of the spawning season, length at maturity, and fecundity are important within these (Blanck and Lamouroux, 2007). Moreover, these traits help describe species' life history strategies. Winemiller and Rose (1992) described three primary life history strategies in fish: opportunistic, equilibrium and periodic (Section 1.5; Chapter 5). Opportunists tend to be small-bodied and mature early, have low juvenile survivorship and are associated with habitats with frequent and intense disturbance (Section 1.5). Equilibrium strategists are small to medium in body size, mature later than opportunists, have low fecundity per spawning event but with high juvenile survivorship through high parental care and are associated with relatively stable habitats (Winemiller and Rose, 1992; Olden et al., 2006; Section 1.5). Periodic strategists are larger, mature even later in life, are highly fecund but with low juvenile survivorship, and are usually associated with seasonal environments (Winemiller and Rose, 1992; Olden et al., 2006; Section 1.5).

Due to the critically endangered status of existing *G. ghorensis* populations, conservation measures are needed to maintain and then enhance their status (Chapter 1). The design of effective conservation measures requires knowledge on the species' biology and ecology, particularly in relation to their life history traits. Chapter 5, working on the age and growth rates of three Jordanian *G. ghorensis* populations,

recorded ages to a maximum of six years old, but with most fish being under three years old. Analysis of monthly length frequency distributions revealed the appearance in samples of young-of-the-year over a protracted period throughout the summer months, suggesting a prolonged spawning season with the possibility of batch spawning. Across the three populations, there was also considerable variability in the life-span and growth rates of individuals, with fish in the most disturbed site being the longest lived and fastest growing (Hamidan and Britton, 2015a). This suggested that these *G. ghorensis* populations have an opportunistic life history with individuals successfully adapting to mildly disturbed habitats (Section 5.4). These inferences however, remain speculative in the absence of data on their reproductive traits. Consequently, the aim of this Chapter was to quantify the reproductive ecology of these three *G. ghorensis* populations using samples collected on a monthly basis over a 12 month period. The objectives were to:

- (1) assess the temporal and spatial variability in *G. ghorensis* reproductive effort;
- (2) use the temporal data on reproductive effort to identify the commencement and duration of the spawning season;
- (3) determine body length: fecundity relationships and identify spatial patterns in fecundity; and
- (4) assess the life history and conservation implications of these outputs.

6.2 Materials and Methods

The fish sampled at each site and month were analysed for: sex ratio, length at maturity, absolute fecundity (F, female fish only) and reproductive effort. For an individual fish, absolute fecundity was determined from a total egg count (i.e. number of vitellogenic oocytes) of a weighed subsample of a weighed ovary and then multiplied up to represent the total egg number of that ovary. The relationship between fecundity (F) and fork length (L_F) was then described by the logarithmic transformation of the power curve

$\log(F) = \log(a) + b \log(L_F)$; where a and b are parameters (Bagenal and Braum, 1978). Length at maturity was calculated from the percentage of mature fish in each 5 mm length class using a modification of the formula of DeMaster (1978). The original formula is based on age classes and so the modification used the 5 mm length classes instead (Trippel and Harvey, 1987). A fish was classed as mature when developed testes or ovaries could be identified in the body cavity. Reproductive effort was assessed by the gonadosomatic index (I_G) of both female and male fish, calculated as gonad weight/(body weight-gonad weight).

The I_G data were used to determine the temporal variation in reproductive effort so that the timing of reproduction and the length of the reproductive season could be deduced, and the role of temperature within this be identified. It was also used to determine how reproductive effort varied between the sites. The fecundity data were used to determine the relationship between fork length and egg production, and to identify how fecundity varied by site. The fish length: fecundity relationship was determined through linear regression following logarithmic transformation of the data. All the other analyses were completed using generalized linear models (GLM). This was because the reproductive effort and fecundity data were not normally distributed, preventing the use of general linear models, and GLM allows the influences of covariates to be controlled in the models, as variables such as fish length tend to significantly influence reproductive trait data. Models testing I_G data used linear distributions and models testing fecundity used Poisson distributions with a log-linear function, as fecundity represented count data of eggs. The variables used in each model are described in the Results section along with their significance. The outputs of each GLM included the mean adjusted values of the groups of dependent variable (as estimated marginal means) and their significance (as pairwise comparisons with Bonferroni adjustment for multiple comparisons). All statistics were completed in SPSS v. 19.0

6.3 Results

The majority of the *G. ghorensis* monthly sub-samples comprised of 15 fish, although numbers were reduced in some months, particularly in winter when sampling conditions were more hostile (Table 6/ chapter 5). The lengths of the sampled fish ranged between 20 and 137 mm, with mean length per month varying through the year and a general trend of decreasing mean length (Table 6). Sex ratios at Site HD and BR were not significantly different from 1:1 (HD: $n = 105$, $\chi^2 = 1.15$, $P = 0.28$; BR: $n = 130$, $\chi^2 = 0.00$, $P = 1.0$), but was dominated by females at Site IB ($n = 152$, 1M: 1.9F, $\chi^2 = 13.92$, $P < 0.01$; Table 6). At sites HD, IB and BR respectively, lengths at 50 % maturity for male were 37.0, 27.7 and 29.3 mm, and for female 47.0, 30.2 and 30.0 mm. These lengths support the previous findings that this species matured below the age 1 (Section 5.3, 5.4). The GLMs testing the effect of month on female reproductive effort (as I_G) with fish length as the covariate were significant at each site (HD: Wald $\chi^2 = 102.1$, d.f. = 10, $P < 0.01$; IB: Wald $\chi^2 = 57.2$, d.f. = 9, $P < 0.01$; BR: Wald $\chi^2 = 64.7$, d.f. = 10, $P < 0.01$). In each model, the effect of month on I_G was significant ($P < 0.01$) whilst fish length was significant at BR and HD ($P < 0.05$) but not at IB ($P = 0.65$). At each site, female I_G varied through the year, with higher values in spring that peaked in May and declined thereafter (Figure 20).

This decline, however, was not consistent, with subsequent increases and decreases in mean I_G during the remainder of the year (Figure 20). Some of these mean values decreased significantly from values in May (e.g. June in Site HD; Table 6/chapter 5) only to increase to non-significant differences once more (e.g. July in Site HD; Table 7). The GLMs of reproductive effort (as I_G) of male fish versus month with fish length as the covariate were significant at each site (HD: Wald $\chi^2 = 119.6$, d.f. = 10, $P < 0.01$; IB: Wald $\chi^2 = 98.3$, d.f. = 9, $P < 0.01$; BR: Wald $\chi^2 = 154.6$, d.f. = 10, $P < 0.01$).

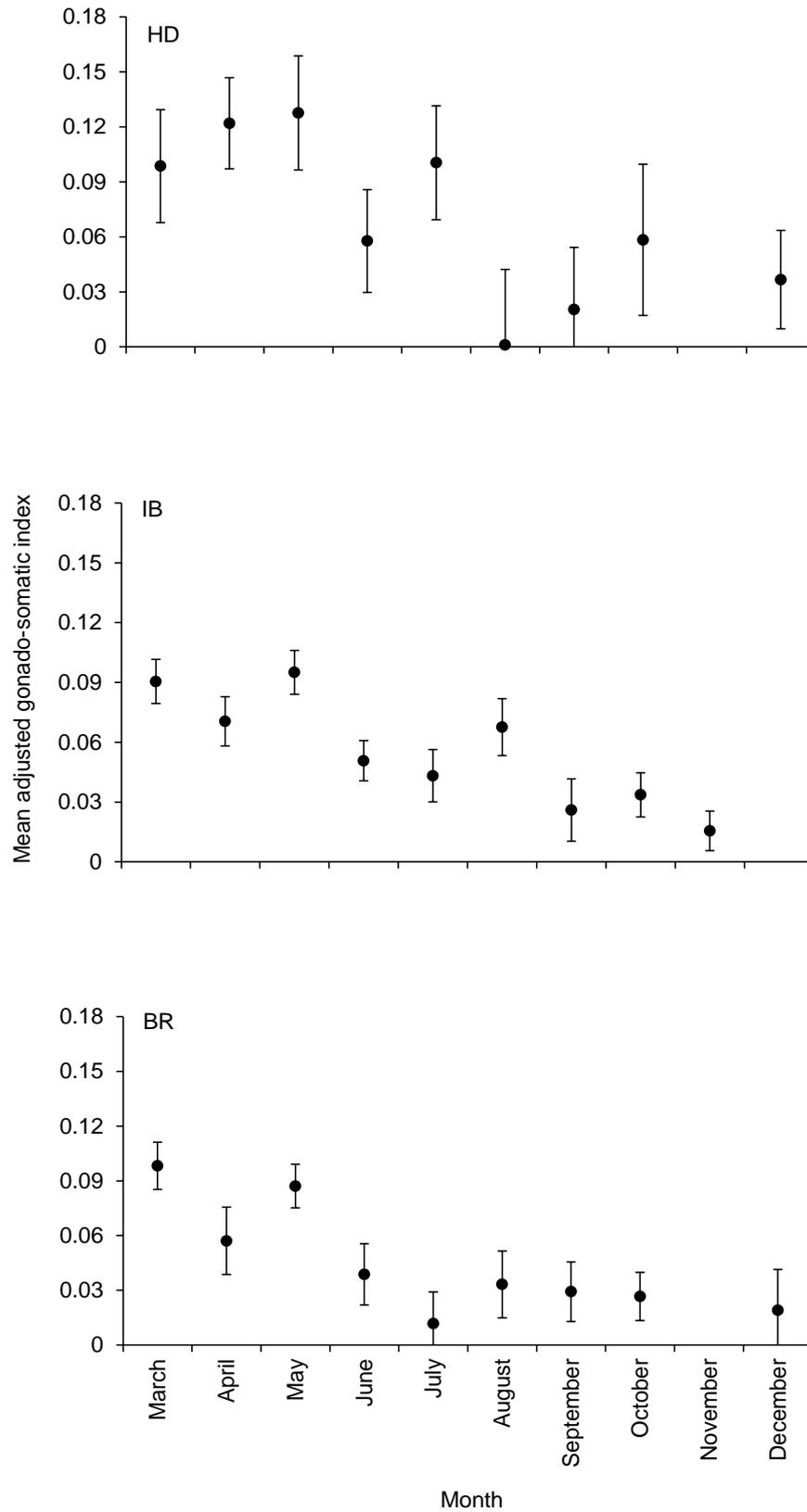


Figure 20 Mean gonado-somatic index (adjusted for the effect of fish length) by length of female *Garra ghorensis* at sites HD, IB and BR. Error bars represent standard error.

Table 7 Pairwise comparisons of mean adjusted gonad-somatic index of female *Garra ghorensis* at each study site between May 2011 and all other months, where values for mean difference are displayed with their standard error, and where * denotes the difference is significant at $P < 0.05$.

	Month	HD	BR	IB
May	February	0.03 ± 0.06	0.09 ± 0.02*	-
	March	0.03 ± 0.06	-0.01 ± 0.02	0.00 ± 0.02
	April	0.01 ± 0.05	0.03 ± 0.02	0.02 ± 0.02
	June	0.08 ± 0.02*	0.07 ± 0.02*	0.04 ± 0.01
	July	0.03 ± 0.03	0.08 ± 0.02*	0.06 ± 0.02*
	August	0.15 ± 0.04*	0.05 ± 0.02	0.03 ± 0.02
	September	0.11 ± 0.03*	0.06 ± 0.02	0.07 ± 0.02*
	October	0.07 ± 0.04	0.06 ± 0.02	0.06 ± 0.02*
	November	0.09 ± 0.02*	0.07 ± 0.03	0.08 ± 0.01*
	December	-	0.07 ± 0.03	-

In each model, the effect of month on I_G was significant ($P < 0.01$), but the effect of fish length was not significant at any site ($P > 0.05$). As with female fish, male reproductive effort was highest in the initial months of the year and peaked around May (particularly in Sites IB and BR; Figure 20). However, unlike the female fish, I_G progressively and significantly declined with each month thereafter (Table 8, Figure 21).

Mean air temperatures by month at the two weather recording stations ranged between 9.8 and 19.2 °C from February through May, and between 21.1 and 25.8 °C from June through October (Figure 7). Comparison of mean I_G and mean monthly air temperature suggested I_G increased as air temperatures increased to 19 °C and then reduced as temperatures exceed 20 °C as the fish commenced spawning (Figure 22). This inference is supported by a GLM testing the effect of mean monthly air temperature (two groups, < 20 and > 20 °C) on I_G with site, sex and fish length being covariates. The model was significant (Wald $\chi^2 = 36.1$, d.f. = 1, $P < 0.01$), with all independent variables significant ($P < 0.05$). It revealed mean adjusted I_G was significantly higher at temperatures below 20°C (that occur before June) (0.07 ± 0.01) than above 20°C (June to October) (0.03 ± 0.01) ($P < 0.01$).

The GLMs testing for the effect of site on reproductive effort with month and fish length as covariates were significant for female fish, but not males (female: Wald $\chi^2 = 39.4$, d.f. = 2, $P < 0.01$; male: Wald $\chi^2 = 1.3$, d.f. = 2, $P = 0.32$; Figure 21). For females, the covariates of fish length and month were also significant ($P < 0.05$), with mean adjusted I_G significantly higher at HD (0.21 ± 0.03) than at IB (0.06 ± 0.02) and BR (0.04 ± 0.03) ($P < 0.01$; Figure 23). The differences in mean adjusted male I_G between the sites were not significant (Figure 23).

Table 8 Pairwise comparisons of mean adjusted gonad-somatic index of male *G. ghorensis* at each study site between May 2011 and all other months, where values for mean difference are displayed with their standard error, and where * denotes the difference is significant at $P < 0.05$.

	Month	HD	BR	IB
May	February	-	0.08 ± 0.01*	-
	March	0.06 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
	April	± 0.01	0.02 ± 0.01	0.04 ± 0.01
	June	0.04 ± 0.01	0.03 ± 0.01*	0.05 ± 0.02*
	July	0.04 ± 0.01	0.05 ± 0.01*	0.06 ± 0.01*
	August	0.05 ± 0.01*	0.06 ± 0.01*	0.06 ± 0.01*
	September	0.03 ± 0.01	0.07 ± 0.01*	0.08 ± 0.01*
	October	0.03 ± 0.01*	0.08 ± 0.02*	0.07 ± 0.01*
	November	-	0.08 ± 0.02*	0.05 ± 0.02
	December	0.03 ± 0.01*	-	0.07 ± 0.01*

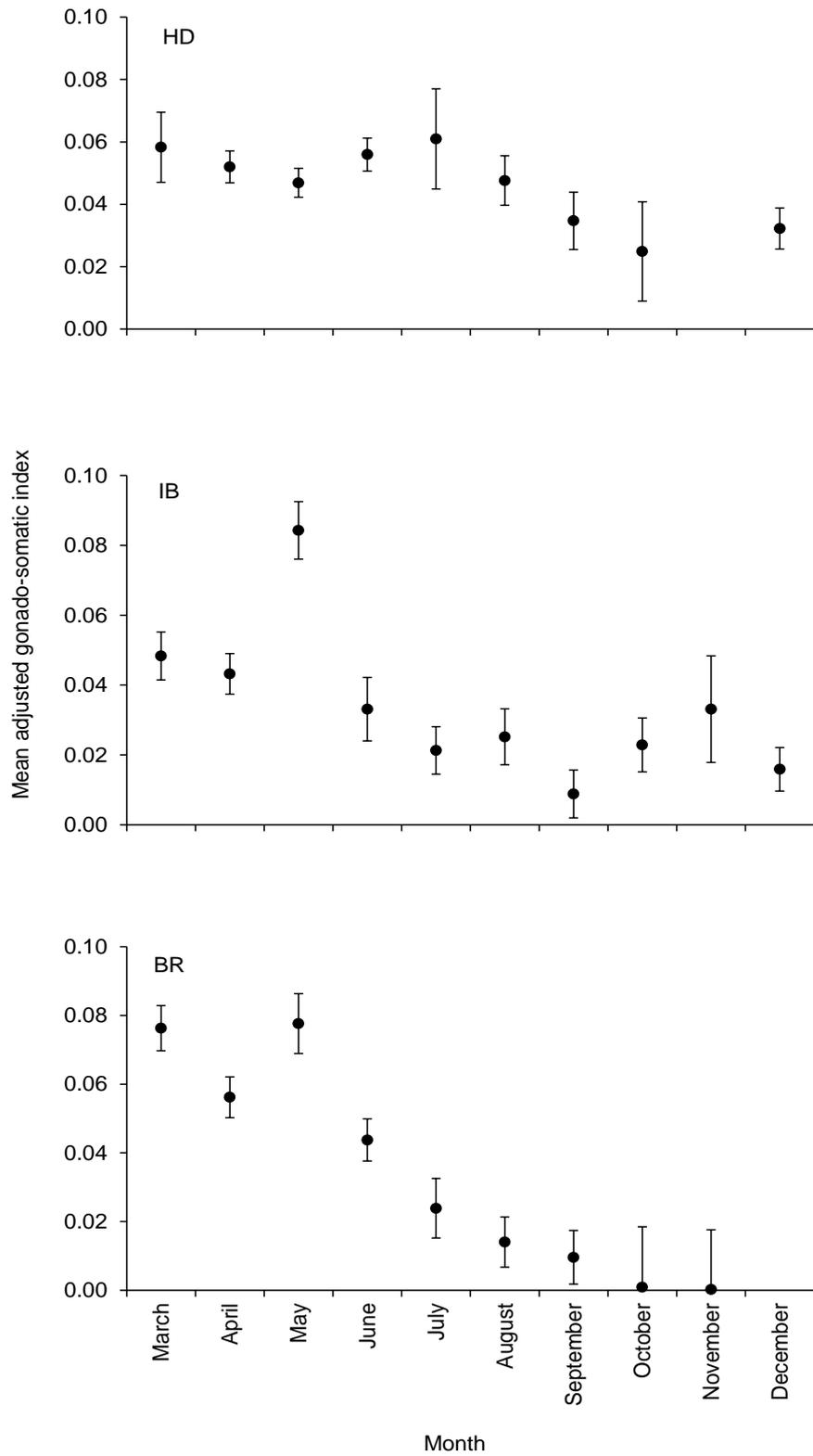


Figure 21 Mean gonado-somatic index (adjusted for the effect of fish length) by length of male *G. ghorensis* at sites HD, IB and BR. Error bars represent standard error.

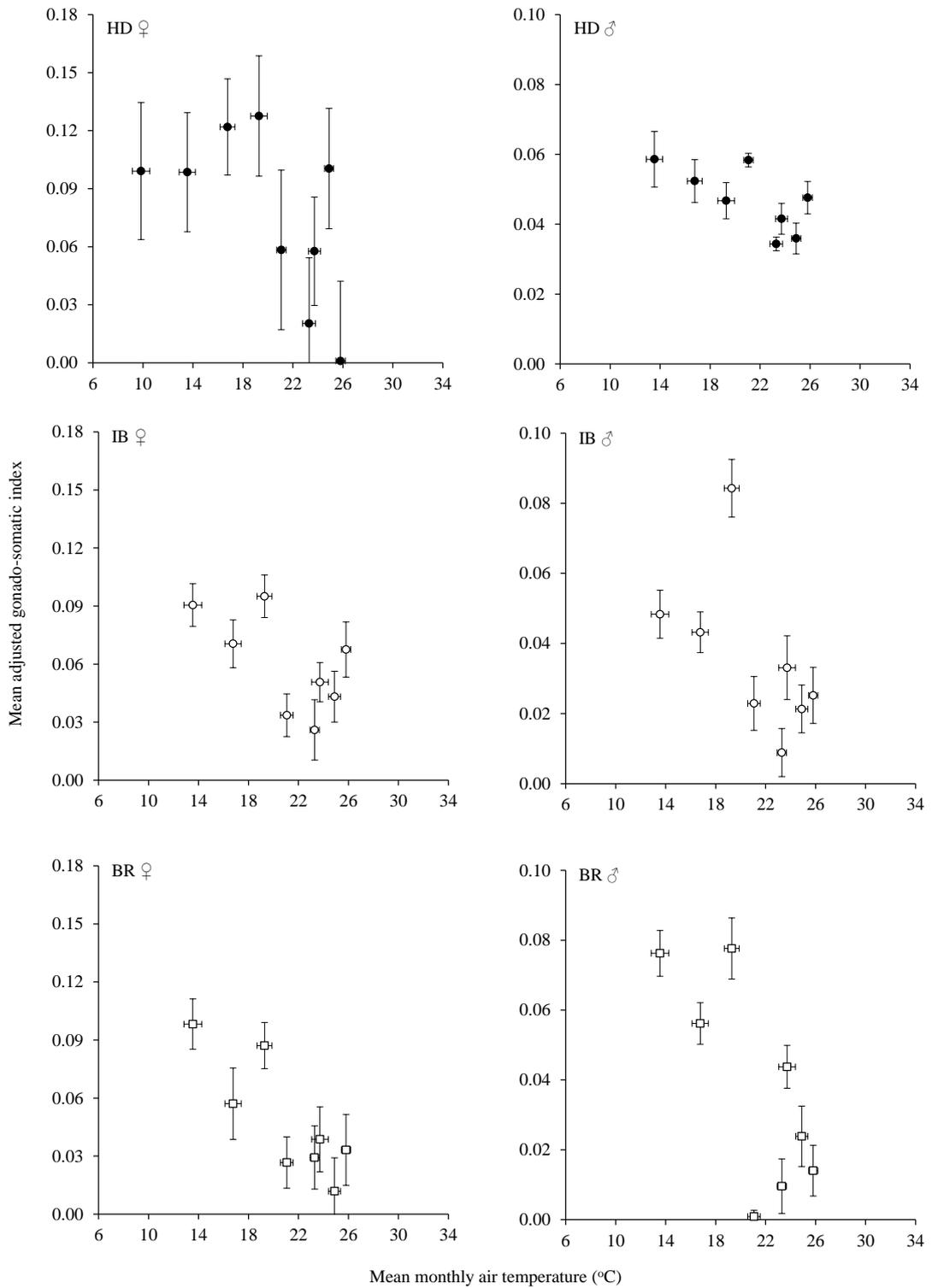


Figure 22 Relationship of mean monthly air temperature at the closest weather recording stations to the sites and mean gonado-somatic index (adjusted for the effect of fish length) at each site (HD, IB and BR) and for each sex (male ♂, female ♀). Error bars represent standard error.

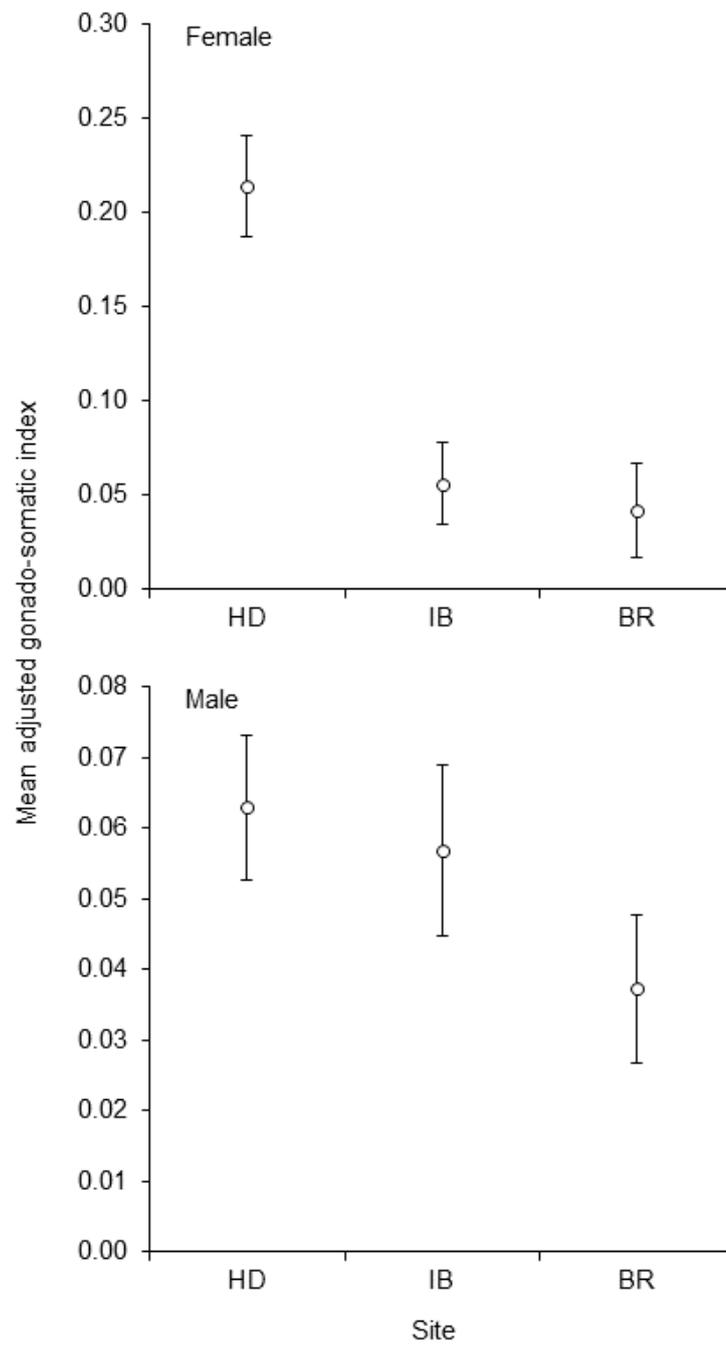


Figure 23 Mean gonado-somatic index (adjusted for the effect of fish length and month) for male and female *G. ghorensis* at sites HD, IB and BR

The relationship between fecundity and fish length only used data for fish sampled prior to June, given the possibility that captured fish from June onwards could have already spawned (Figure 20; Table 9). The relationship between fish length and fecundity was significant at all sites (linear regression; Table 9). Comparison of fecundity between sites also only used data from fish sampled prior to June. The GLM was significant (Wald $\chi^2 = 11.50$, d.f. = 2, $P < 0.01$), with the effect of site and fish length also significant ($P < 0.01$), but not month ($P = 0.84$). Mean adjusted fecundity was significantly higher at HD (435 ± 44 ripe oocytes) than at both IB (232 ± 38 ripe oocytes) and BR (242 ± 38) ripe oocytes ($P < 0.01$). Mean adjusted fecundity between IB and BR was not significantly different ($P > 0.05$).

Table 9 Relationship between fish length and fecundity per site according to linear regression and where fecundity estimates are derived from $\log(F) = \log(a) + b \log(L_F)$. Values in parentheses are the lower and upper 95 % confidence levels. Error around the mean is standard deviation.

Site	Mean length (mm)	Length range (mm)	Log(a)	b	R ²	F statistic	P
HD	83.8 ± 14.1	48.2 - 113.4	0.12 (-3.80 to 4.05)	1.33 (0.46 to 2.20)	0.31	F _{1,22} = 10.10	< 0.01
BR	64.4 ± 17.2	33.0 - 103.9	-1.24 (-7.05 to 4.56)	1.51 (0.09 to 2.92)	0.20	F _{1,20} = 4.95	0.04
IB	55.9 ± 13.3	32.1 - 84.2	-3.91 (-7.75 to -0.07)	2.19 (1.24 to 3.13)	0.48	F _{1,25} = 22.64	< 0.01

6.4 Discussion

The reproductive traits of the three *G. ghorensis* populations revealed early maturation and relatively high reproductive effort. Considering their small body size and limited life-span (generally below 3 years; Hamidan and Britton, 2015a; Chapter 5), this suggests that the populations are expressing an opportunistic life history (Winemiller and Rose, 1992), a strategy associated with frequently disturbed habitats (Olden et al., 2006). The *G. ghorensis* populations occurred in habitats with high temperatures (maximum daily air temperatures exceeded 40 °C in July and August) and minimal rainfall (i.e. low flows) in summer, and low temperatures (minimum daily air temperatures regularly fall below 0 °C in January) and relatively high rainfall (which can result in episodic floods) in winter. These conditions suggest a relatively harsh environment, especially when allied to the anthropogenic disturbances apparent at the sites, particularly HD where impoundments have replaced much of the fast, shallow water with slower, deeper sections. In disturbed environments, the life history traits of fish are theorised as predisposing fish to extinction risk as they determine their vulnerability to disturbances (Reynolds et al., 2005; Pool and Olden, 2012). Desert fishes with ‘slow’ life histories (e.g. large body sizes, long life-spans and delayed maturity) tend to have greater frequencies of local extirpation and so higher extinction risk (Olden et al., 2008). By contrast, desert fishes with ‘fast’ life histories and opposite suite of traits have greater resilience to extirpation and extinction. Consequently, the life history traits of *G. ghorensis* present in these three sites in Jordan suggest their populations have some inherent resilience to extirpation through their life history traits and opportunistic strategy.

The overall risk of extirpation and extinction of fishes due to habitat loss and invasions is variable according to not only the traits of the species concerned but also their ability

to adapt to the new conditions through trait plasticity (McKinney, 1997; Olden et al., 2006, 2008). Thus, whilst the opportunistic traits of *G. ghorensis* appear to generally provide resilience against extirpation and even extinction, this is coupled with their ability to adapt to changing conditions through trait plasticity. Site HD was the most disturbed site through impoundments and the presence of invasive *O. aureus*, yet individuals in this *G. ghorensis* population were the longest-lived and fastest growing (Section 5.3, 5.4; Hamidan and Britton, 2015a), and had significantly higher reproductive investment compared to fish in the other sites in terms of both female gonado-somatic index and fecundity. This indicates there is little evidence that invasive *O. aureus* were impacting *G. ghorensis* spawning success, an impact that has generally been recorded in other sympatric fishes (Doupé et al., 2008). Thus, these outputs suggest that in mild hydrological disturbance, the plasticity of *G. ghorensis* reproductive traits provide some resilience and persistence to populations.

The female reproductive effort data suggested that *G. ghorensis* commenced spawning in May and June; when tested against the air temperature data, outputs suggested initial spawning occurred at air temperatures of approximately 20 °C. Following this initial spawning event, spawning was asynchronous, with the gonado-somatic index of females being variable throughout the summer months and was then largely unrelated to air temperature. Observations of the ovaries of these fish undertaken during fecundity estimates between February and May also revealed that in addition to oocytes ripening in response to temperature increases (and presumably also to changes in day length and flow regime), there remained numbers of undeveloped eggs present. Indeed, batch and asynchronous spawning is a feature of many cyprinid fishes (e.g. Rinhard and Kestemont, 1996; Nunn et al., 2002; Oyugi et al., 2011). Although *G. ghorensis* mainly spawned in June, some females protracted their spawning throughout the summer months. This was also supported by the appearance of young-of-the-year fish (YOY) in

samples throughout this period (Hamidan and Britton, 2015a). Similar to their trait plasticity, this reproductive strategy potentially provides increased resilience to disturbance through production of YOY over a protracted period that ensures some will survive to winter even if there is a disturbance in the early summer that would otherwise result in high YOY mortality (Hamidan and Britton, 2015a).

In summary, the completion of these analyses on the reproductive traits of *G. ghorensis* in the three sites confirmed that their life histories were aligned to the opportunistic strategy of Winemiller and Rose (1992). This should provide their populations with some resilience to changing environments that stems from their trait plasticity, relatively fast growth, early maturity and high fecundity.

Chapter 7. Diet and trophic niche of the endangered fish *Garra ghorensis* in three Jordanian populations

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7.1 Introduction

Disturbed environments are often more vulnerable to the invasion of non-native species, as their often more generalist traits and high capacity for adaptation enable them to take advantage of the modified conditions (McKinney, 1997; Marvier et al., 2004). Whilst this combination of habitat disturbance and invasion increases the risk of local native fish populations being extirpated and endemic fishes becoming extinct (Olden and Poff, 2005), this risk varies between species according to their traits, and their ability to adapt to the modified environment and co-exist with invasive species (Olden et al., 2006 2008; Hamidan and Britton, 2015b). Consequently, understanding how endemic and threatened species respond to modified environments and interact with invaders is integral to impact assessment and the design of conservation strategies to ameliorate and/ or mitigate impact (Fausch et al., 2006).

Jordan is considered the fourth water poorest country in the world (Denny et al., 2008) and its rivers are increasingly being impounded in order for water to be allocated for domestic use and ground water recharges (Chapter 1, 4). This has altered previously fast-flow inland shallow rivers with high longitudinal connectivity to river sections that are slower flowing, substantially deeper and often disconnected from their adjacent sections (Hamidan and Mir, 2003). Allied to this has been the release of non-native fish, usually for enhancing fish productivity in aquaculture using cichlid species such as *Oreochromis niloticus* and *O.aureus*. These introductions have also often occurred in habitats where endemic fish species are also present, such as *G. ghorensis* (Hamidan, 2004; Hamidan and Britton, 2015a,b; Chapter 1).

In situations where an endemic species of high ecological value is under increasing habitat impoverishment and is assessed as endangered, then research into their status and ecology can be justified where the studied populations are shown to be locally

abundant in preliminary surveys and so sampling will not affect the sustainability of the extant populations (Leunda et al., 2008). Consequently, work on *G. ghorensis* so far in its constricted range in Jordan range has focused on the expression of its life history traits in disturbed environments, revealing that even in the most disturbed environment studied, their ability to reproduce, have a relatively long life span (for the species) and grow relatively fast was not constrained (Hamidan and Britton, 2015a,b; Chapter 5, 6). This chapter builds on this work through assessment of *G. ghorensis* diet, with a focus on assessing feeding relationships across three contexts: in allopatry, and in sympatry with a native fish (*Capoeta damascina*) and in sympatry with an invasive fish (*Oreochromis aureus*). Given their level of conservation threat then each context could only be studied at a single site and thus replication of contexts was not possible (Chapter 1, 4). The objectives were to:

- (1) assess the diet and trophic niche breadth of *G. ghorensis* and the other fishes through stomach contents analysis;
- (2) assess the long-term trophic niche breadth and trophic interactions of *G. ghorensis* and the invasive *O. aureus* and the native *C. damascina* through stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$); and
- (3) assess the effect of co-existing fishes on *G. ghorensis* diet and feeding relationships, and in relation to their endangered status.

7.2 Materials and Methods

The diet and feeding relationships were analysed by two main methods: stomach contents analyses and stable isotope analysis. The former utilised all sampled fish collected at each site (Chapter 2, section 2.2), whereas stable isotope analysis was conducted on a smaller proportion of these sampled individuals. Note that logistical

constraints in the field prevented collection of invertebrate samples for analysis of food availability and so no comparison could be made in relation to diet composition versus food availability.

7.2.1 Stomach contents analyses

Stomach contents analyses were conducted through examining the contents of the intestine using dissecting microscope ($\times 7$ to $\times 45$ magnification). During the analyses, the number of empty intestines was noted. Food items were identified to the lowest taxonomic group possible, according to the remains and their ability to provide enough information for a positive identification. They were then grouped into the following categories according to the level at which they were identified: Spirogyra (algae), Gastropoda, Coleoptera, Odonata (as nymphs), Diptera, Ostracoda, Chironomid larvae, Formicidae, zooplankton (primarily *Daphnia* sp.), unidentified plant material, detritus, juvenile fish, fish eggs, unidentified invertebrate (where remains were encountered, such as wings and legs, that did not allow further identification) and digested material (tissues and structures in the stomach that could not be assigned a more specific category).

For each fish species at each site, the stomach contents data were presented in two ways. Firstly, the data were combined for all months, providing a broad overview of the food items consumed by the fishes. Secondly, the data were split by season according to the mean monthly air temperatures calculated from data from the two metrological stations in the vicinity of the sampling sites (Figure 5; Chapter 2). Accordingly, data were combined for each species and site for December, January, and February (winter: < 12 °C); March, April and May (spring: 12 to 20 °C); June to September (summer, > 20 °C), and October and November (autumn, 12 to 20 °C) (Figure 24). The stomach content data were then used in the following indices and metrics. The vacuity index

(%Iv) was calculated as the percentage of empty stomachs to the total number of stomachs examined (Hyslop 1980). Numerical analysis of food items was applied after Windell and Bowen (1978). Food items were represented as ‘frequency of occurrence’ (%Fi) and the ‘relative abundance’ of a given prey item (Ai).

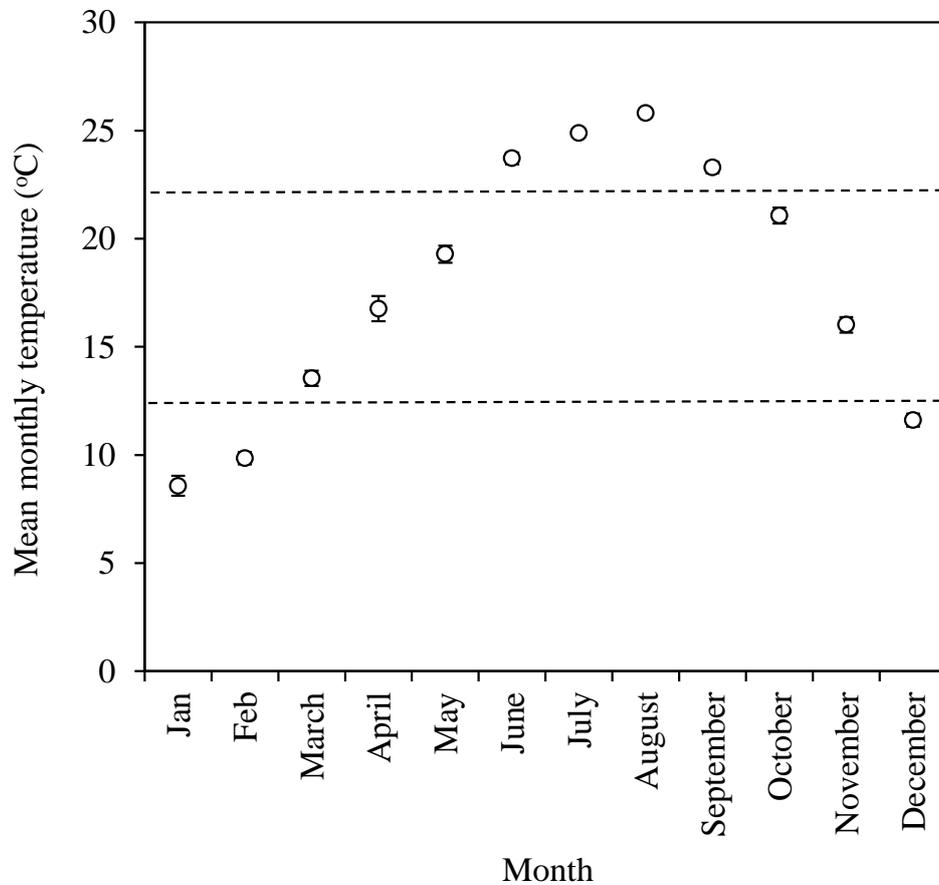


Figure 24 Mean air temperature per month recorded from the two recording station close to the study area (Figure 5/ Chapter 2). Error is standard error; horizontal lines mark the temperature bands used to combine monthly data by season (< 12 °C; 12 to 20 °C; > 20 °C).

Frequency of occurrence, defined as the percentage of stomachs in which that prey occurred, was calculated as: $\%Fi = (Ni/N) \times 100$, where: Ni = Number of intestines containing item i and N = the total number on non-empty intestines. The relative abundance of prey items, defined as the percentage of total stomach contents in all stomachs that comprised of that prey item, was calculated as: $Ai = (\sum Si / \sum S_t) \times 100$, where S_i = the stomach contents (numbers) composed of prey i , S_t = the total stomach contents (Number) of all stomachs in the entire sample (Total number of all stomach items). Note that for Ai calculations, detritus, spirogyra and digested material were unable to be included due to the requirement for numerical data. For analyses of Fi and Ai by season, all food items were included in calculations but only the principal food items were selected for reporting for the sake of brevity. Trophic niche breadth was calculated from the stomach content data to test the species food specialisation, according to Levins (1968):

$$B = \frac{1}{\sum p_j^2}$$

Where B = Levins' measure of niche breadth, P_j = Proportion of the individuals found in our using resource state j , or fraction of items in the diet that are of food category j (Estimated by: N_j / Y) ($\sum p_j = 1.0$), N_j = Number of individuals found in or using resource stat j , $Y = \sum N_j$ = Total number of individuals sampled.

Niche overlap was calculated as a percentage, according to:

$$P_{jk} = \left[\sum_{i=1}^n (\text{minimum } p_{ij}, p_{ik}) \right] 100$$

where P_{jk} = percentage overlap between species j and species k ; p_{ij} = proportion resource i is of the total resources used by species j ; p_{ik} = proportion resource i is of the total resources used by species k ; and n = total number of resource states. Percentage

overlap is the simplest measure of niche overlap to interpret because it is a measure of the actual area of overlap of the resource utilization of the two species. This overlap measure was used by Schoener (1970) and has been labelled the Schoener overlap index (Hurlbert, 1978). Abrams (1980) recommends this measure as the best of the measures of niche overlap. Niche breadth and niche overlap were both calculated with EcoMethodology 7.2 software package.

7.2.2 Stable isotope analysis

Stable isotope analysis provided a longer-term perspective of the diet of the fishes as it represents assimilated diet (up to 6 months where dorsal muscle is used; Grey 2006), where $\delta^{15}\text{N}$ is the indicator of trophic level and $\delta^{13}\text{C}$ is the indicator of energy source (Cucherousset et al., 2012). The analyses were completed using replicate samples of the putative fish-food resources (benthic macro-invertebrates, back-swimmers and algae) collected during sampling, with triplicate samples used where possible. The dorsal muscle samples were from a random selection of up to 30 fish per species and site, taken from samples collected in October and November 2011, i.e. from fish at the end of their growth season when their stable isotope data from their dorsal muscle would be representative of their assimilated diet during the preceding summer months (Perga and Gerdeaux, 2005). All samples were dried at 50 °C for 48 hours before being sent to the Cornell Isotope Laboratory for analysis (Cornell University, New York, USA). Here, they were ground into a homogenous powder, approximately 0.5 mg weighed out into a tin cup, with the actual weight recorded using a Satorius MC5 microbalance. The nitrogen and carbon isotopes were then analysed, using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer. The outputs were values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each individual fish and their putative food resources. As the tissues and macro-invertebrates had been preserved in 70 % ethanol then there was the possibility that some consequent shifts in stable isotope signatures had occurred. For example, Kelly et al. (2006)

suggested that although ethanol did not significantly enrich $\delta^{15}\text{N}$ in tissues of Arctic charr *Salvelinus alpinus*, some significant changes were detected in $\delta^{13}\text{C}$. However, given the absence of species-specific stable isotope correction factors for *G. ghorensis*, *C. damascina* and *O. aureus*, the fish data were used uncorrected, on the basis that $\delta^{15}\text{N}$ was unlikely to have altered significantly (Kelly et al., 2006), and any correction factor for shifts in $\delta^{13}\text{C}$ would have to be applied equally over the three species and thus would not alter their trophic positions and trophic niche sizes relative to each other. Similarly, whilst the macro-invertebrate samples can also shift slightly in their stable isotope signatures following preservation in ethanol (e.g. Sarakinos et al., 2002), then given they were used to only assess relative trophic position then they were also used in their uncorrected form.

To assess differences in the trophic position (TP) of each fish per species and site (i), the $\delta^{15}\text{N}$ data were converted to TP using the formula $[(\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{baseline}}) / 3.4] + 2$, where $\text{N}_{\text{baseline}}$ is the mean $\delta^{15}\text{N}$ of the putative food resources. The items used as putative resources at each site were assessed by the outputs of both the stomach contents data and the extent of their differentiation with the fish isotope values (*cf.* Figure 25). The trophic position data were then tested using a generalized linear model (GLM), as the data were not normally distributed. In the model, trophic position was the dependent variable, the interaction of site and species was the independent variable, and differences in trophic positions between species and sites were tested by pairwise comparisons with Bonferroni adjustment for multiple comparisons. The same model structure was also used to test for differences between species and sites in $\delta^{13}\text{C}$. To determine the trophic niche width of each species at each site according to assimilated diet, the metric ‘standard ellipse area’ was used (SEA_c) (Jackson et al., 2011; Jackson et al., 2012). These ellipses are based on the distribution of individuals in isotopic space as an estimate of each species core trophic niche using the ‘siar’ package (Jackson et al.,

2011; Parnell et al., 2010) in the 'R' computing programme (R Core Development Team 2012). The subscript 'c' in 'SEAc' indicates that a small sample size correction factor was used, as sample size tended to be below 20 per species (Jackson et al., 2011). Where *G. ghorensis* was present in sympatry then the extent of their overlap in trophic niche with the other fish species present was quantified (%). For the trophic niche calculations, the data were not combined between years so that any temporal differences could be identified.

7.3 Results

Site IB: Allopatric Garra ghorensis

The mean length of the *G. ghorensis* at Site IB was 89.5 ± 20.3 mm. Of the 165 fish analysed, 54 had empty stomachs ($Iv = 18\%$). Frequency of occurrence of diet indicated that detritus, digested material and spirogyra were the most frequently encountered food categories in their stomachs, with Odonata nymphs the only macro-invertebrate present in their diet at a frequency $> 1\%$ (Table 10). In contrast to the other populations studied, no gastropods were encountered in the stomachs (Table 10). Detritus was the most important dietary items between Spring and Autumn, and Spirogyra was most important in Winter (Table 11). The relative abundance data indicated that Odonata species were an important food item, particularly in the spring, although values were relatively high in all seasons (Table 12, 13). The overall dietary niche breadth was 0.34, being the highest recorded across the three *G. ghorensis* populations, with this also generally reflected in the seasonal data (Table 14) where niche breadth was highest in winter (Table 14). The stable isotope data of 28 allopatric *G. ghorensis* (mean length: 54.1 ± 15.8 mm) indicated that their mean trophic position was 3.21 ± 0.05 and their standard ellipse area (as a measure of trophic niche size) was 2.86‰^2 (Figure 25).

Site BR, Garra ghorensis in sympatry with native Capoeta damascina

The mean length of the *G. ghorensis* used in the analyses at Site BR was significantly smaller than *C. damascina* (54.11 ± 20.3 mm and 85.03 ± 28.7 mm respectively; ANOVA: $F_{1,325} = 125.81$, $P < 0.01$). Of 158 *G. ghorensis* stomachs analysed, 69 were empty ($Iv = 43$ %), whereas of 168 *C. damascina* stomachs analysed, 14 were empty ($Iv = 8$ %). For both species, the frequency of occurrence indicated that detritus was the most frequently encountered food item, with Spirogyra, Gastropoda and plant material also present, albeit in differing frequencies between them (Table 10). The main contrast in these data between the species was in Odonata nymphs, which were not recorded in any *G. ghorensis* stomachs but were recorded in 26 % of *C. damascina* (Table 10). Coleoptera, Diptera, juvenile fish and zooplankton were also present in *C. damascina* diet, although their frequency of occurrence was < 2 % (Table 10). The dominance of detritus in the diet of both fishes was also apparent in each season, with frequency of occurrence peaking in autumn for *C. damascina* (83 %) and in winter for *G. ghorensis* (67 %, Table 11). For *C. damascina*, Odonata nymphs were most prominent in diet in summer (50 %; Table 11). The relative abundance data emphasized the difference in the diet of the sympatric fishes related mainly to the Odonata in *C. damascina* in all seasons (Table 12, 13). Although the relative abundance of Gastropoda was high in *G. ghorensis*, especially in spring, this was partly due to the relatively low number of numerical food items in their stomachs (Tables 10, 12, 13).

The overall niche breadth of *G. ghorensis* was relatively low when compared to *C. damascina* (0.10 and 0.24 respectively; Table 14). Their overall niche breadths overlapped by 72 %. Seasonally, the smallest calculated niche breadth for *G. ghorensis* occurred in summer when they were mainly consuming detritus, and was highest in spring, when the contribution of spirogyra and Gastropoda increased (Table 13, 14). A value for *G. ghorensis* in autumn was unable to be calculated due to the low diversity of

items taken, indicating a very small niche. The niche breadth of *C. damascina* was also considerably higher than *G. ghorensis* in all seasons except spring. The highest dietary overlap with *C. damascina* occurred in winter and varied between 41 and 72 % across the seasons (Table 14).

Stable isotope analysis was completed for 15 *G. ghorensis* (mean length: 41.6 ± 15.8 mm) and 17 *C. damascina* (mean length: 69.5 ± 22.1 mm). The differentiation between the fish and their putative food resources suggested that macro-invertebrates were important assimilated items compared with algae (mean $\delta^{15}\text{N}$: all fish: 16.75 ± 0.13 ‰; macro-invertebrates: 12.87 ± 0.40 ; algae: 3.84 ± 0.56 ; Figure 25). There were significant differences between the species for trophic position (GLM: Wald $\chi^2 = 76.70$, d.f. 4, $P < 0.01$); *G. ghorensis* being higher (3.32 ± 0.07 vs. 3.03 ± 0.05 ; $P < 0.01$). Although the trophic position of *G. ghorensis* was higher than at Site IB, the difference was not significant (0.11 ± 0.08 , $P > 0.05$). The GLM testing differences in $\delta^{13}\text{C}$ between the species was significant (Wald $\chi^2 = 335.14$, d.f. 4, $P < 0.01$), with *G. ghorensis* $\delta^{13}\text{C}$ depleted compared to *C. damascina* (-23.27 ± 0.37 versus -21.74 ± 0.28) (Figure 25). The trophic niche sizes according to SEAc revealed that the niche of *C. damascina* (4.18 ‰^2) was considerably larger than *G. ghorensis* (2.48 ‰^2), with a niche overlap of 8 % (Figure 25).

Site HD, Garra ghorensis in sympatry with invasive Oreochromis aureus

The mean length of analysed *G. ghorensis* at Site HD was 80.32 ± 20.4 mm and *O. aureus* was 129.7 ± 27.8 mm, with these differences not being significant (ANOVA: $F_{1,254} = 0.41$, $P > 0.05$). Of the 123 *G. ghorensis* stomachs analysed at the site 22 were empty ($Iv = 17$ %), with 47 of 141 *O. aureus* stomachs also being empty ($Iv = 33$ %). Frequency of occurrence data indicated both species were feeding strongly on algae (as *Spirogyra*), with detritus also frequently occurring in their stomachs (Table 10).

Gastropoda and Odonata nymphs were also taken frequently by *G. ghorensis*, with this much less apparent in *O. aureus* (Table 10). Instead, the *O. aureus* stomachs contained low frequencies of fish eggs, Formicidae, Chironomid larvae and juvenile fish (< 6 %), all of which were absent in *G. ghorensis* (Table 10). Seasonally, the frequency of occurrence of Gastropoda and Odonata nymphs to *G. ghorensis* diet was always considerably higher than for *O. aureus*, with these items well represented in all seasons (except autumn) for *G. ghorensis* (Table 11). In summer, both fishes had high frequencies of spirogyra in their diet (Table 11). The relative abundance of Gastropoda was high for both species (Table 12), although the effect was seasonal, as they were present less frequently in spring in both fishes (Table 12). The relative abundance of Odonata was high in *G. ghorensis*, especially in spring, but was absent in *O. aureus*, whereas chironomid larvae and fish eggs were relatively abundant in *O. aureus* but not *G. ghorensis* (Table 12, 13). The dietary niche breadth of *G. ghorensis* was always higher than *O. aureus*, irrespective of season, although there was overlap in their diets (42 to 74 %; Table 14). Niche breadth of *G. ghorensis* was lowest in summer, when items including zooplankton were absent in diet (Table 11, 14).

Stable isotope analysis was completed for 16 *G. ghorensis* (83.6 ± 14.6 mm) and 12 *O. aureus* (75.9 ± 13.5 mm). Mean $\delta^{15}\text{N}$ of all fish was 7.82 ± 0.17 ‰, macro-invertebrates 5.67 ± 0.52 ‰ and algae 3.54 ‰, suggesting both macro-invertebrates and algae had some contribution to assimilated diet (Figure 25). The outputs of the GLMs revealed the difference in mean TP between *G. ghorensis* and *O. aureus* was significant (TP: *G. ghorensis* 3.72 ± 0.07 , *O. aureus* 3.45 ± 0.06 , $P = 0.03$), but was not for $\delta^{13}\text{C}$ (1.15 ± 0.50 , $P > 0.05$) (Figure 25). The trophic position of *G. ghorensis* at this site was significantly higher than at Sites BR and IB ($P < 0.01$ in both cases). Trophic niche size according to SEAc revealed *G. ghorensis* and *O. aureus* had similar sized trophic niches (4.33 ‰² and 4.00 ‰² respectively) and overlapped by 27 % (Figure 25).

Table 10 Frequency of occurrence of the food item categories of each fish species in each sampling location (all data).

	IB	BR		HD	
Food Item	<i>G. ghorensis</i> (n = 165)	<i>G. ghorensis</i> (n = 158)	<i>C. damascina</i> (n = 168)	<i>G. ghorensis</i> (n = 123)	<i>O. aureus</i> (n = 141)
Detritus	37.8	67.4	73.4	21.8	50.0
Digested material	28.8	20.2	22.7	12.9	1.1
Unidentified invertebrate	8.1	3.4	7.1	4.0	3.3
Spirogyra	20.7	4.5	14.3	52.5	58.5
Gastropoda	0.0	3.4	3.9	24.8	7.4
Plant material	2.7	4.5	14.3	0.0	9.6
Odonata nymph	8.1	0.0	26.0	19.8	0.0
Zooplankton	0.9	0.0	1.3	2.0	0.0
Coleoptera	0.0	0.0	0.6	0.0	0.0
Diptera	0.0	0.0	0.6	0.0	0.0
Juvenile fish	0.0	0.0	0.6	0.0	3.2
Chironomid larvae	0.9	0.0	0.0	0.0	4.3
Formicidae	0.0	0.0	0.0	0.0	1.1
Fish eggs	0.0	0.0	0.0	0.0	5.3
Ostracoda	0.9	0.0	0.0	3.0	0.0

Table 11 Frequency of occurrence of selected food items of each species in each sampling location by season (n = number of guts examined).

Site	Species	Season	n	Items				
				Detritus	Spirogyra	Gastropoda	Odonata	Zooplankton
IB	<i>G. ghorensis</i>	Spring	45	48.7	18.9	0.0	8.1	2.7
		Summer	69	28.8	11.5	0.0	5.7	1.9
		Autumn	30	50.0	28.0	0.0	7.1	0.0
		Winter	30	11.8	35.3	0.0	11.8	17.7
BR	<i>G. ghorensis</i>	Spring	44	27.3	13.6	9.1	0.0	0.0
		Summer	54	42.0	0.0	1.0	0.0	3.0
		Autumn	24	20.0	0.0	0.0	0.0	0.0
		Winter	36	67.4	4.5	3.4	0.0	3.4
	<i>C. damascina</i>	Spring	45	83.1	9.5	7.1	11.9	0.0
		Summer	60	73.2	17.8	3.5	50.0	7.10
		Autumn	19	83.3	11.1	5.5	27.7	11.1
		Winter	44	52.6	15.8	7.9	7.9	15.8

Site	Species	Season	n	Items				
				Detritus	Spirogyra	Gastropoda	Odonata	Zooplankton
HD	<i>G. ghorensis</i>	Spring	44	18.9	40.5	24.3	35.1	2.7
		Summer	29	6.9	68.9	31.0	13.7	6.9
		Autumn	5	40.0	40.0	40.0	0.0	40.0
		Winter	45	37.1	45.7	20.0	8.6	2.9
	<i>O. aureus</i>	Spring	45	22.2	33.3	2.2	4.0	2.2
		Summer	49	36.3	69.6	3.0	0.0	0.0
		Autumn	11	100.0	50.0	16.6	0.0	0.0
		Winter	36	63.3	46.7	13.3	0.0	6.7

Table 12 Relative abundance (as mean number of items per stomach) of food item categories of each fish species in each sampling location (all data).

	IB	BR		HD	
Food Item	<i>G. ghorensis</i> (n = 165)	<i>G. ghorensis</i> (n = 158)	<i>C. damascina</i> (n = 168)	<i>G. ghorensis</i> (n = 123)	<i>O. aureus</i> (n = 141)
Unidentified invertebrate	29.0	9.7	6.6	1.2	3.7
Gastropoda	0.0	87.1	8.3	44.4	26.7
Odonata nymph	41.9	3.2	81.0	35.3	0.0
Zooplankton	3.2	0.0	1.2	1.6	0.0
Coleoptera	0.0	0.0	1.8	0.0	0.0
Diptera	0.0	0.0	0.6	0.0	0.0
Juvenile fish	0.0	0.0	0.6	0.0	3.2
Chironomid larvae	3.2	0.0	0.0	0.0	42.9
Formicidae	0.0	0.0	0.0	0.0	0.9
Fish eggs	0.0	0.0	0.0	0.0	22.6
Ostracoda	9.7	0.0	0.0	16.7	0.0

Table 13 Relative abundance (as mean number of items per stomach) of selected food items of each species in each sampling location by season

Site	Species	Season	n	Items		
				Gastropoda	Odonata	Zooplankton
IB	<i>G. ghorensis</i>	Spring	45	0.0	75.0	25.0
		Summer	69	0.0	38.4	46.2
		Autumn	30	0.0	33.3	66.6
		Winter	30	0.0	42.9	28.6
BR	<i>G. ghorensis</i>	Spring	44	100.0	0.0	0.0
		Summer	54	33.3	0.0	66.6
		Autumn	24	0.0	0.0	0.0
		Winter	36	87.1	3.2	9.7
	<i>C. damascina</i>	Spring	45	0.0	83.3	0.0
		Summer	60	1.7	93.1	3.4
		Autumn	19	4.3	86.9	4.4
		Winter	44	66.0	20.0	6.0
IB	<i>G. ghorensis</i>	Spring	45	0.0	75.0	25.0
		Summer	69	0.0	38.4	46.2
		Autumn	30	0.0	33.3	66.6
		Winter	30	0.0	42.9	28.6

Site	Species	Season	n	Items		
				Gastropoda	Odonata	Zooplankton
HD	<i>G. ghorensis</i>	Spring	44	23.9	72.8	1.1
		Summer	29	80.6	19.3	0.0
		Autumn	5	14.6	0.0	58.4
		Winter	45	66.0	20.0	6.0
	<i>O. aureus</i>	Spring	45	1.3	0.0	0.0
		Summer	49	9.1	0.0	81.8
		Autumn	11	100	0.0	0.0
		Winter	36	85.7	0.0	10.7

Table 14 Trophic niche breadth of each species in each site, and overall and by season, according to the methods of Levins (1968) and calculated from stomach contents data. Values in parentheses are the extent of the niche overlap between the fishes at that site in that season. Note at Site BR in autumn, there were insufficient items in diet to enable calculation.

Site	Species	Overall	Spring	Summer	Autumn	Winter
IB	<i>G. ghorensis</i>	0.34	0.47	0.54	0.47	0.61
BR	<i>G. ghorensis</i>	0.10 (71.6%)	0.58 (41.2%)	0.25 (62.6 %)	-	0.20 (72.2%)
	<i>C. damascina</i>	0.24	0.16	0.42	0.38	0.48
HD	<i>G. ghorensis</i>	0.20 (54.4%)	0.59 (41.7%)	0.423 (57.7%)	1.00 (39.7%)	0.44 (73.8%)
	<i>O. aureus</i>	0.13	0.44	0.343	0.580	0.26

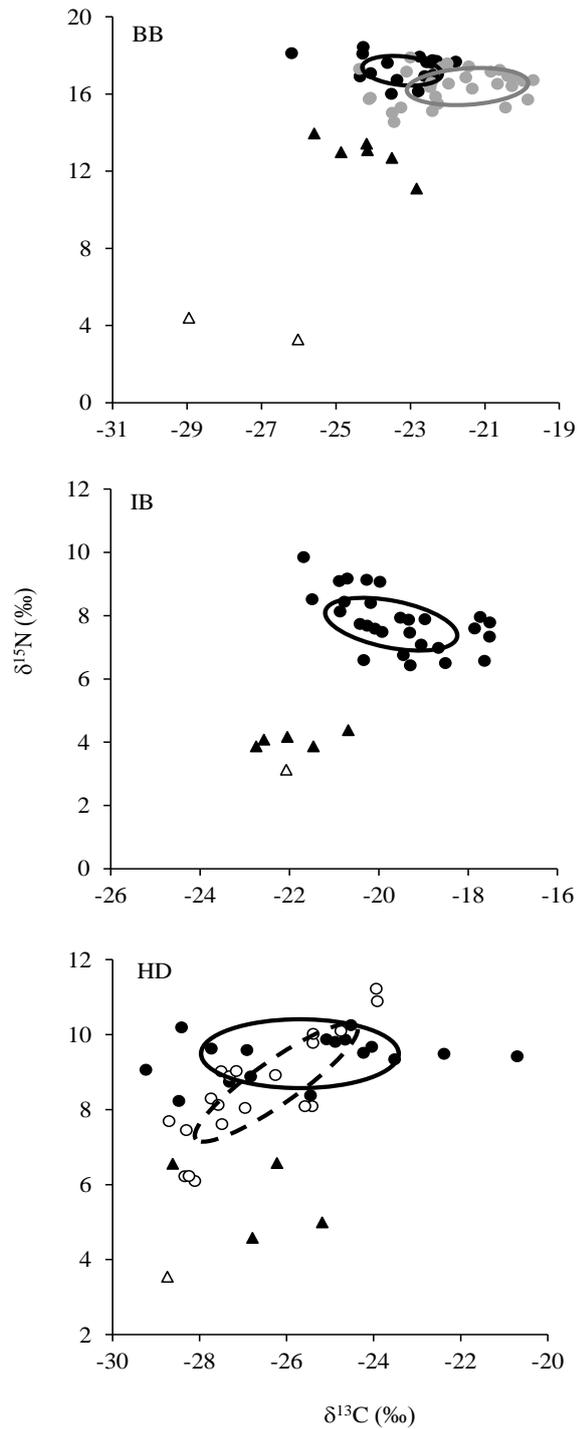


Figure 25 Stable isotope biplots of *Garra ghorensis* (black circles), *Capoeta damascina* (grey circles) and *Oreochromis aureus* (clear circles), and their trophic niche breadth as measured by standard ellipse area (SEAc) where solid black line = *G. ghorensis*, grey line = *C. damascina*, and dashed line = *O. aureus*. Black triangles are macro-invertebrate samples and clear triangles are algal samples. Note differences in scales on the axes.

7.4 Discussion

Garra ghorensis is endangered throughout its range, restricting the number of populations that could be used to study aspects of their ecology that would then inform conservation strategies. Here, only three populations were used, each representing a different context (allopatric, sympatric with a native fish and sympatric with an invasive fish) without replication. Correspondingly, whilst the data outputs indicate the extent to which the *G. ghorensis* populations were responding to the different contexts, the lack of replication might inhibit the identification of more general dietary patterns in relation to aspects such as invasions. Nevertheless, the outputs did suggest that in general, the populations of *G. ghorensis* had a greater proportion of empty stomachs than sympatric fishes and were heavily reliant on feeding on algae (spirogyra) and detritus that whilst are of low nutritive value, are rarely limiting (Persson, 1983). In stomachs, contributions of animal material to diet appeared low, although the stable isotope data suggested they made more important contributions to assimilated diet than the stomach contents data suggested. Nevertheless, these data suggest that all of the fishes used in the study were specialising on detritus and phyto-plankton.

The reliance of the fishes on detritus and spirogyra in the stomach contents might represent a dietary preference over animal material, or might be reflective of a low availability of animal material at each site for the fishes, especially given the stable isotope outputs. Also, given the variation in site characteristics, then food availability might also have differed between sites and influenced diet choice. However, as the temporal and spatial abundance of these items were not measured at each site, this could not be investigated further. Nevertheless, the relatively high contribution to diet of algae and detritus compared to other resources in all sites is important as it can be speculated that these would be least likely to be limiting of all the resources consumed (Persson,

1983). This is important, as all outputs suggested the diet of *G. ghorensis* and the invasive *O. aureus* had some degree of overlap (stomach contents analysis: 54 %; stable isotope analysis: 27 %). Moreover, when invasive populations establish following an introduction, niche-based competition theory predicts that where there is dietary overlap between species that results in competition then the subordinate competitor will shift to alternative food resources, reducing their trophic niche but with partitioning promoting their coexistence (Sepulveda et al., 2012). Consequently, that this was not evident here suggests there was no requirement for shifting to alternative food resources, i.e. despite the resource sharing, competition was not evident and so did not alter their diet (Guo et al., 2014). Indeed, the *G. ghorensis* population that was sympatric with *O. aureus* comprised of relatively fast growing, highly fecund individuals (Hamidan and Britton, 2015a,b) and so there was no supporting evidence that there were detrimental ecological consequences arising from sharing food resources.

The complementary use of stomach contents and stable isotope data to study the diet and feeding relationships of fishes is now routine, including for investigating feeding interactions of invasive and native fishes (e. g. Leunda et al., 2008; Cucherousset et al., 2012). Their complementary use is beneficial as although stable isotope analysis has many benefits, including use of relatively small sample sizes and provision of long – term insights into diet composition, it also has a series of limitations. These limitations include issues relating to fish size, as fish diet is strongly related to ontogeny, thus differences between species could just be an artefact of differences in fish length and thus also their gape size (Cucherousset et al., 2012). Indeed, the complementary use of stable isotope analysis and stomach contents analysis can often provide contrasting outcomes. For example, Locke et al. (2013) used stomach contents, stable isotopes and trophically transmitted parasites to analyse the diet of pumpkinseed *Lepomis gibbosus*, and revealed each method provided virtually unrelated information, with no significant

correlation in the importance of prey items across the methods. In this chapter, however, there were some consistent outcomes in the combined use of stomach contents and stable isotopes. For example, they both indicated that *C. damascina* had a larger trophic niche than *G. ghorensis* at Site BR and these niches overlapped. The stomach contents data did, however, suggest that the niche breadth of *G. ghorensis* was higher than *O. aureus* in all seasons, with this was not apparent in the stable isotope data that suggested a relatively similar niche breath. This difference might relate to the stomach contents data being used seasonally, whereas the stable isotopes were used to primarily analyse the assimilated diet at the end of the growth season.

In addition, the extent of assimilation into muscle tissue of items such as detritus, plant material and spirogyra, all common items in the stomachs, might be relatively low due to issues including their difficulty of digestion, resulting in their prolonged presence in the intestine (and so potentially a high contribution to stomach contents data) but low assimilation (and so potentially a low contribution to stable isotope data) (McCutchan et al., 2003).

This low assimilation of plant material was supported by the differentiation in stable isotope values between fish and their putative resources that suggested macro-invertebrates might have been more important in terms of assimilated diet than suggested by the stomach contents data. Thus, these animal resources might have been making relatively important energetic contributions to the fishes and so been important for maintaining their population stabilities.

In summary, the outputs of the two dietary analytical techniques suggested that whilst there were some spatial differences in the diet of *G. ghorensis*, they were primarily consuming detritus and algae in each site, with this also being the case for the sympatric fishes, with the low proportion of animal material consumed being important for their

assimilated diet. Whilst this resulted in some overlap in the diet of *G. ghorensis* and the sympatric fishes, it was cautiously deduced that the fishes were unlikely to have been competing for this resource and so the endangered status of *G. ghorensis* was unlikely to be resulting from negative feeding interactions with an invasive fish.

Chapter 8. Discussion

8.1 Overview

The research studied the endangered fish species *G. ghorensis*, starting with analysis of their biogeographic origin and phylogeography, and assessment of their current distribution in the context of potentially reduced habitat availability in the last decade. The research then focused on three populations across a gradient of disturbance, where disturbance was in relation to both habitat alteration and the presence of an invasive fish. Analyses were completed on their life history traits and their feeding relationships. The aim of this final chapter is to thus provide a synthesis of the results and identify on-going conservation threats to the species. Context is also provided in relation to studies on other desert fishes, impacts of invasive species and relevant aspects of ecological theory.

8.2 Phylogeography and distribution

The analyses of the mitochondrial DNA of the *Garra* genus reported in Chapter 3 were designed to determine the biogeographic origin and phylogeography of *G. ghorensis*. This was important for a number of reasons. Firstly, surveys on the species completed in recent years (*cf.* Chapter 4) indicate a very restricted spatial distribution and thus it was important to understand how their current distribution related to patterns that might have been more evident in the past. Secondly, this region of the Middle East has been subject to a series of studies that have provided contrasting perspectives on the biogeographic origin of species such as *G. ghorensis*, with some suggesting it is major biogeographical crossroads (Banarescu, 1992; Coad, 1996a), whilst others suggest it is a centre of speciation (Por, 1985). Finally, as genetic analyses can indicate where a population has been subjected to historical changes in the physical and biotic environment (Feliner, 2014; Mossop et al., 2015), then it was able to highlight the potential importance of the

proto-lakes of Samra and Lisan in driving current distribution patterns. Thus, the genetic work, although only completed on mitochondrial DNA, was extremely important in providing context on the origin and distribution of *G. ghorensis*.

It was apparent from the genetic results that the biogeographic origin of *G. ghorensis* was not the Arabian Peninsula, despite their morphological similarity to *G. tibanica* and contrary to the hypothesis suggested by the work of Krupp (1982). Instead, the phylogenetic data indicated that as *G. ghorensis* is sharing a common ancestor with *G. rufa* then their origins had to be the basins of the Mediterranean and Persian/Arabian Gulf, as per Goren and Ortal (1999) and Gorshkova et al. (2012). Importantly, these outputs also suggested that the absence of *G. ghorensis* in the southern Dead Sea basin, as detected in Chapter 3, was not necessarily due to alterations in habitat and hydrology of these wadis in recent times, but instead were most likely a legacy of the proto-lakes of Samra and Lisan. This was because these results suggested that the common ancestor of *G. jordanica* and *G. ghorensis* was likely to have originally occupied the coastal waters of Samra Lake, but the subsequent development of the variant salinities Lisan Lake split the populations of *G. jordanica* and *G. ghorensis* around the Mujib River (Figure 8, 9, 11). Their complete disconnection occurred as the salinity of Lisan Lake reached lethal levels. The *Garra* populations were then limited to the wadis in their respective regions where there was sufficient high freshwater flow. Consequently, the fish species and their distributions that were present in Lake Samra underwent some alterations in distribution and development as the salinity of Lake Lisan increased. Thus, the absence of fishes from the southern Dead Sea area, as detected in Chapter 3 where a number of sites were sampled and no *G. ghorensis* were recorded (Table 3), might not be due to the influence of human activities in contemporary times. Instead, it could be the legacy of the salinity of Lake Lisan extirpating populations in historical

times, thus removing freshwater fish species that would otherwise have been endemic to the area.

These results on the origin of *G. ghorensis* and their biogeographic constraints are important given the work on their contemporary distribution completed since 2002. Hamidan and Mir (2003) originally sampled six sites and detected *G. ghorensis* in all of them. In 2010, these surveys were repeated, with an additional eight sites also fished. The results indicated that there was minimal change in their distribution, with individual fish captured at each of the original six sites of Hamidan and Mir (2003). However, across the new sites fished, only one new population of *G. ghorensis* was detected. Although there could arguably be a case that this lack of new populations detected was the result of populations being recently extirpated by human disturbance, the lack of baseline data from earlier times, plus the issues already outlined in relation to Lisan Lake, suggest this is unlikely. Instead, it can be argued that the distribution map of Figure 14 is a strong representation of their contemporary distribution that has primarily resulted from temporal changes in the geology and water chemistry of the region over a very prolonged period.

The importance of bringing these results from Chapter 3 and 4 together is that they help interpret the on-going issues associated with the endangered status of *G. ghorensis*. They highlight the natural limits on their distribution, thus enabling more focus on recent changes within this range. The results of Chapter 4 suggested that in the 2000s, there was little change in their distribution in this range, at least in relation to those sites in which it was logistically possible to sample, and highlighted the populations that were locally abundant and present in either allopatry or in sympatry. These populations were then important for highlighting those populations that could withstand regular sampling for subsequent analysis of their diet and life history traits (Chapters 5 to 7).

The surveys of 2010 were also important in highlighting that although the distribution of *G. ghorensis* had not altered, the physical characteristics of the sites sampled had, such as through construction of water impoundments. Although their recording was largely subjective in the study, given logistical constraints (Table 3), it was highlighted in Chapter 3 that these alterations potentially result in a loss of longitudinal connectivity in these wadis. This could then lead to habitat and population fragmentation, and potentially weakening the ability of individuals to adapt to changes in their environment, such as climate change (Section 8.5).

8.3 Life history traits

Chapters 5 and 6 studied aspects of the life history traits of *G. ghorensis* across three sites, focusing initially on age and growth rates (Chapter 5) and then on reproductive traits (Chapter 6). These traits are fundamentally important in the context of the conservation of the species as, more generally, they are capable of highlighting fishes that are vulnerable to extirpation via their suite of life history traits. For example, Olden et al. (2006) summarised the three life history strategies that represent the endpoints of a triangular continuum arising from trade-offs among the three basic demographic parameters of survival, fecundity, and onset and duration of reproduction (Section 1.5; Figure 3). These life history strategies of periodic, opportunistic and equilibrium are also strongly indicative of the environments in which the species inhabit. For instance, an opportunistic strategist, usually a small-bodied fish of early maturation, low fecundity per spawning event, and low juvenile survivorship, typically inhabit highly disturbed and unpredictable environments (Section 1.5). By contrast, an equilibrium strategist, usually a small to medium bodied fish with moderate maturation age, low fecundity per spawning event, and high juvenile survivorship, typically inhabits constant environments (Olden et al., 2006).

These strategies also align to the r and k life history strategies (Table 15). These strategies all exist in a continuum, with species not necessarily at a fixed position on this, but varying by populations according to abiotic and biotic constraints (Figure 3). Work on the desert fishes of the Colorado River has strongly indicated that it is the r -strategists that are most resilient to withstanding aspects of environmental change, where relatively high plasticity in their traits enables them to compensate for modified conditions by altering their growth and reproductive output, such as through faster growth enabling earlier maturation and then higher reproductive outputs (e.g. Olden et al., 2006; Mims et al., 2010; Section 1.5).

Table 15 Typical characteristics of r -selected (opportunistic) and k -selected (equilibrium) fish populations (from Pianka 1994)

Characteristic	r -selected	k -selected
Maturation	Early in life	Relatively late in life
Life span	Short	Medium to long
Mortality rate	High	Low
Number of progeny per reproductive episode	Many	Few
Number of reproductive episodes per lifetime	Usually one	Often several
Timing of first reproduction	Early in life	Late in life
Egg/ progeny size	Small	Large
Parental care	None	Often extensive

By contrast, *k*-selected, equilibrium species are highly vulnerable to extirpations and extinctions (Olden et al., 2006; Section 1.5). For *G. ghorensis*, the results of Chapters 5 and 6 indicated that they rarely lived for more than 3 years, with growth rates that were relatively rapid early in life but slowed thereafter, and had high reproductive effort (e.g. high gonado-somatic index and fecundity) across a relatively short reproductive season. They are also a gravel spawning species, with no parental care. Thus, these results indicate that *G. ghorensis* utilised a r-selected life history strategy, with these traits also aligned to the opportunistic life history strategy of Winemiller and Rose (1992). Whilst there was variability in these traits between sites, this variability indicated an intensely r-selected strategy at IB and BR, where the habitat and environmental conditions (e.g. seasonal flow rates) were likely to be more variable than HD due to their lack of impoundment. At HD, it appeared that whilst a r-selected strategy was still being utilised by *G. ghorensis*, it was less intense than at IB and BR, as the altered habitat result in more stable conditions. Thus, across the range of *G. ghorensis*, whilst there will be some variability in their traits according to the habitat and environmental conditions, their life history traits will always consist of early maturity (<3 years), high relative fecundity and short body lengths (<180 mm), i.e. a r-selected strategy. The studies completed on the Colorado River (*cf.* Chapter 1) therefore indicate that this is highly advantageous by providing *G. ghorensis* populations with some resilience to tolerate some aspects of environmental changes.

The results of both Chapters 5 and 6 also indicated that these growth and reproductive traits for *G. ghorensis* were plastic. Site HD was interpreted as the most altered hydrologically, with reduced flows and increased depths, whereas the other two sites were relatively undisturbed, with more natural flow regimes and habitats (Chapter 2). Growth rates were significantly higher and life spans longer at HD, with greater reproductive investment, despite being relatively altered from the apparently preferred

habitat of the species. Indeed, the results suggested that the reduced flows might have provided the population at HD with greater energy for investment in growth and reproduction, with the more stable hydrological regime resulting from impoundment providing some distinct advantages in terms of greater longevity and reproductive output. However, it is worth to say, that this kind of habitat alteration, can put more impact on *G. ghorensis* due to invasion, and changing in water chemical and physical parameters, so *G. ghorensis* invest more in reproduction and growth, that is way the subject of flow impact on length and production is recommended for further studies. These results are highly important as they indicate that, providing the hydrological disturbance at sites where *G. ghorensis* is present still enables the completion of their life cycle (e.g. there is still sufficient spawning substrate, nursery areas, and adult refugia and feeding areas) then their populations can withstand some anthropogenic disturbances. Importantly, these results are contrary to Propst et al. (2008), who suggested that to conserve endangered desert fishes in habitat altered environments requires the restoration of the natural flow regimes in which they evolved. Arguably, in the face of human development in securing water supplies in arid regions, such perspectives might be considered as highly unrealistic. Thus, by arguing that some habitat alteration is acceptable but stipulating that the habitat requirements of all life stages must still be met could help ensure that *G. ghorensis* populations can be maintained throughout their range.

Notwithstanding these results and interpretations, it should also be noted that these studies were completed on three populations that were locally abundant. Had they also included some sites and populations that were under a greater risk of extirpation, such as from more extreme hydrological changes, then potentially some altered interpretations might have resulted. However, destructive sampling of vulnerable populations of endangered species would be have substantial ethical implications and be

contrary to subsequent conservation efforts. Nevertheless, given the successful use of scales for age and growth analysis, it could be argued that some age and growth analyses could be completed on some vulnerable populations. This is because scales can be collected using non-destructive methodologies and information on the age structure populations can help indicate recent reproduction and recruitment failures.

8.4. Trophic ecology

The trophic ecology of fishes can help interpret aspects of their status by identifying their feeding relationships and where there is potential for the adverse effects of competition to develop. Across the three sites that were studied, *G. ghorensis* was present in allopatry and then in sympatry with *C. damascina* (native fish) and *O. aureus* (invasive cichlid). For *G. ghorensis*, stomach contents analyses consistently showed detritus and algae were prominent food items in their diets, with low dietary contributions of animal material. Calculations of trophic niche size indicated their niches were small compared with *C. damascina* and overlapped by over 70 %, whereas it was larger than *O. aureus*, but still overlapped by 54 %. Complementary use of stable isotope analysis indicated greater contributions of animal material to assimilated diet, but also indicated that the trophic niche breadth of *C. damascina* was higher than *G. ghorensis*, with some overlap (26 %). Similarly, the trophic niche of *G. ghorensis*, as indicated by stable isotope analysis, was also larger than *O. aureus*, and did overlap (27 %). These results are important as when in sympatry with the other fishes, they highlight the potential for *G. ghorensis* to compete for the shared food resources.

Trophic niche based competition theory predicts that where there is inter-specific niche overlaps between species that results in competition, then the competitors will shift to alternative food resources, reducing their trophic niche size and potentially diverging in resource use, with this partitioning promoting their coexistence (Sepúlveda et al., 2012; Hamidan, et al., 2016, and Chapter 7). This partitioning was not evident here, given the

dietary overlaps between *G. ghorensis* and *C. damascina* and *O. aureus*. This was interpreted in Chapter 7 as being because there was no requirement for partitioning; that is, despite the trophic niche overlaps, competition was not evident via reduced and/or partitioned niches and so the fishes did not alter their diet (Guo et al., 2014). This might be related to the stomach contents data that suggested that *G. ghorensis* were heavily reliant on feeding on algae (spirogyra) and detritus, as these are rarely limiting in freshwaters. Moreover, they indicate that despite the presence of a highly invasive fish, there was no evidence trophically that this was having an ecological impact on *G. ghorensis*. This is again important in the context of the conservation of their populations, as they suggest they are capable of co-existing with invasive species. However, detailed future studies on food availability and seasonal food availability can provide wide understanding.

8.5 Conservation threats

8.5.1 Invasive species

At the global level, freshwater fishes are among the most widely introduced aquatic animals, with at least 625 freshwater fish species now having been introduced outside of their natural range (Gozlan et al., 2010). A primary driver of these introductions is the gaining of socio-economic benefits via aquaculture (Gozlan, 2008; Gozlan et al., 2010). It was for this reason that cichlid species have been introduced into Jordan, with species including *O. aureus* being farmed. Although potentially solving food supply issues and providing socio-economic benefits, where these fishes escape into the wild and develop invasive populations then ecological consequences can be incurred (Gozlan et al., 2010). A strong example is the release of the Nile perch *Lates niloticus* into Lake Victoria, East Africa, which has been implicated in the decline, and even extirpation, of over 200 haplochromine cichlid fishes (Barel et al., 1985; Ogutu-Ohwayo, 1990; Kische-

Machumu et al., 2012). Other examples include hybridisation of the already threatened native trout species (e.g. *Oncorhynchus apache* and *Oncorhynchus gilae*) with the introduced rainbow trout *Oncorhynchus mykiss* in North America (Vitule et al., 2009) and hybridisation of crucian carp *Carassius carassius* with introduced goldfish *Carassius auratus* and *C. carpio* in Britain (Hänfling et al., 2005). Additionally, *C. carpio* have also been shown to increase water turbidity and decrease aquatic macrophytes and zooplankton in invaded water bodies (Lougheed, 1998). Regarding invasive cichlids, *Oreochromis mossambicus* is listed by the IUCN on the list of the world's worst 100 invaders (ISSG, 2006).

In the field studies of Chapters 5 to 7, however, there was little evidence of detrimental impacts of *O. aureus* on aspects of *G. ghorensis* ecology, although it should be noted that these studies were not necessarily designed to be impact assessments. They do, however, highlight that detecting ecological impacts can be difficult and that in some cases, ecological impacts might indeed be minimal (Gozlan, 2008; Gozlan et al., 2010). Notwithstanding, from a conservation perspective, given the impacts recorded in many regions of the world regarding non-native fishes used in aquaculture such as invasive cichlids and *C. carpio*, including the Middle East (e.g. Coad, 1996b; Zak et al., 2014) then it would be unwise to discount invasive fishes generally as a long-term conservation threat to *G. ghorensis*. Moreover, with increasing impoundment of rivers in their range then the lentic conditions required for the aquaculture of fishes such as *Oreochromis niloticus* will continue to increase. The introduction of inappropriate fish species for aquaculture could thus be managed through implementation of risk assessment, such as the Fish Invasiveness Scoring Kit (FISK; Copp et al., 2009). This should help prevent the use of inappropriate fishes that will develop invasive, high impacting populations if they escape into the wild.

8.5.2 *Habitat disturbance and loss*

As *G. ghorensis* is a rheophilic species, requiring flowing water and gravels for spawning substrate, then it can be argued that the most destructive habitat alteration in the wadis would be a total shift from lotic to lentic conditions caused by complete impoundment and reservoir construction. Indeed, such impoundments generally have the capacity to result in large losses of endemism, alter thermal regimes downstream of the impoundment and increase the risk of invasion by non-native species (Johnson et al., 2008; Olden and Naiman, 2010; Lehner et al., 2011). In the case of *G. ghorensis* blocking the flow regime and the continuous cleaning of the gravelly habitats removed a considerable space of spawning grounds either by removing the substrate (gravels) manually, or by covering them with sediments that used to be naturally cleaned off by the annual flow events.

Activities such as water abstraction, whether from surface or ground waters, can alter the hydrology of rivers, reducing flow rates, especially during dry periods in summer (Benejam et al., 2010). This abstraction can then have negative ecological consequences for river biota, including the fish assemblage (Benejam et al., 2010; Lange et al., 2014). However, where data are available to ensure that the abstraction leaves sufficient flows for the fish community to remain sustainable (Cowx et al., 2012) then this flow reduction should not necessarily imperil the populations. Data requirements on flow rates of fishes and the ecological consequences of flow reductions can be assessed through packages such as PHABSIM (Physical Habitat Simulation; Moir et al., 2005; Beecher et al., 2010), although this is designed for salmonid fish communities (Louhi et al., 2008). It does, however, enable environmentally sustainable flows to be implemented that enable some abstraction without affecting the long-term sustainability of fish populations. The natural flow of river is particularly important in the case of *G. ghorensis*, it cleans the substrate of sediment, creates nursery sites (river pockets)

beside the fast flowing rivers, and the effect of oxygenates the eggs over the spawning grounds.

The long-term drivers of these threats to the physical habitats of the wadis in the past 20 years are likely to from water shortages that result in more extreme measures being taken to increase water security for society and industry, such as damming that recreates reservoirs. Unlike schemes currently at Site HD that deepen the channel and reduce flows but still enables the *G. ghorensis* population to be sustained, a switch to a completely lentic habitat would eliminate their access to spawning areas. Moreover, the potential for prolonged periods of extreme weather events such as drought, which result in increased water shortages, are predicted to increase under climate change (Section 8.5.3).

8.5.3 Climate change

General climate change projections for Jordan consistently suggest that air temperatures will increase by up to 2 °C by 2050, with warming effects being stronger in the summer than in the winter (Ministry of the Environment, 2013). Site scale predictions (i.e. at smaller spatial scales) then suggest increased temperatures between 1 and 4 °C and, more crucially, decreased precipitation by between 15 and 60 %. This is predicted to be accompanied by extreme events, such as flash floods, intense rains, snow storms and drought (Ministry of the Environment, 2013). Given that Jordan is already among the driest countries in the world, with water scarcity a major issue that constrains economic growth and development, then this is a potentially major socio-economic and political issue. Available water resources per capita are already falling, while water demand and the water shortage will drastically increase in the future due to population growth and anticipated socio-economic development. Moreover, groundwater levels have already dramatically declined, suggesting groundwater exploitation has been unsustainable (Ministry of the Environment, 2013). Water management in the country is supply-based

and although there have been significant improvements in water-supply infrastructure, there remains a critical and serious supply-demand imbalance, exacerbated by the recent influx of displaced people from neighbouring countries due to political instability in the region (Ministry of the Environment, 2013).

These climate change predictions suggest that these existing water supply-demand problems will only worsen and this will put greater stress on surface waters. Rahel and Olden (2008) suggest that in general, the outcomes of such climate change predictions will be altered stream flows and thermal regimes, increased water salinity and the increased development of water supply schemes to satisfy societal demands. Assuming these outcomes occur in the Dead Sea basin then it would suggest that the current freshwater habitats of *G. ghorensis* will become more prone to more extreme low flows in summer, when temperature increases will also result in warmer waters with concomitant changes in water chemistry, such as decreased dissolved oxygen levels. There will be potentially elevated flows in winter, with these often having deleterious effects on juvenile fish numbers (Gorski et al., 2011); alternatively, these waters could compensate the low level of riverine water caused by ground water and surface water abstraction. Indeed, similar hydrological outcomes have been predicted for many rivers across the world (Döll and Zhang, 2008). For example, large decreases in Q_{95} (i.e. the flow rate that is exceeded in a particular river for 95 % of the time) are predicted in summer for rivers in Great Britain, with this likely to have substantial consequences for river biota, including fishes (Johnson et al., 2008).

The suggestion of Rahel and Olden (2008) that increased water scarcity will result in increased engineering to secure water supplies would then suggest that there will be increasing pressure on the wadis of the Dead Sea basin for impoundments, as these should provide more stable surface water supplies for the surrounding communities and agricultural activities. It has already been discussed that some habitat disturbance of this

nature can be tolerated by *G. ghorensis*, with their trait plasticity providing some adaptive capacity to the new conditions. However, should these schemes become more extreme, with development of permanent lentic conditions in place of the prevailing lotic conditions, then this is likely to result in the local extirpations of the *G. ghorensis* populations due to loss of spawning areas. Moreover, in these lentic waters, the risk of non-native species being used in aquaculture is increased, raising further concerns on their potential for resulting in irreversible changes to the native biota (Rahel and Olden, 2008; Section 8.5.1).

8.6 Recommendations

The following recommendations are proposed that all aim to provide long-term sustainability in the populations of *G. ghorensis*. They seek to maintain the spatial distribution of the species in their natural range and within this, for populations to have the ability to form locally abundant populations.

1. Maintenance of the Red Listing of *Garra ghorensis* as an endangered species.

As *G. ghorensis* has a spatially limited distribution, with this in a region of political and climate uncertainty, the anthropogenic pressures on their populations will remain and, most likely, increase. Therefore, recognition that *G. ghorensis* is an endangered species will provide some wider recognition that their populations are going to remain imperiled in the face of anthropogenic pressures, climate change and biological invasions for a long time to come.

2. Develop sustainable water supply strategies that provide adequate water supplies for societal needs but also sustainable flows for the *G. ghorensis* populations; and

3. Education of water managers, engineers and planners over the need to conserve *G. ghorensis* populations.

Given that the major threat to the long-term sustainability of *G. ghorensis* is the maintenance of river flows and habitat availability in which to complete their lifecycle, then schemes to secure water supplies must take their populations into account. Any shift in habitats to entirely lentic conditions would lead to population extirpations and, ultimately, extinction. Abstraction of ground waters that reduce surface water flows to minimal levels, especially in drought, needs to be avoided. Thus, the maintenance of, for example, sufficient flows and spawning gravels must be incorporated into all water supply schemes and the need to conserve *G. ghorensis* population be embedded into environmental impact assessments (EIAs). This will require education of relevant industries and individuals, including planners, engineers, water managers and those involved in completing EIAs.

4. Integrate *G. ghorensis* as an important, resilient and endemic species in the local public awareness programme that is implemented by the Royal Society for the Conservation of Nature (RSCN) in general and around the protected areas within the range of *G. ghorensis* distribution, in particular the local communities around Fifa Protected Area a 23 sq. kilometres mixed saline and salt plain where the two southernmost wadis (Khneizereh and Fifa) drain.

5. Following the steps of the conservation project of the endemic Azraq Killifish *Aphanius sirhani* in Jordan. It is recommended to integrate the “story of conservation of *G. ghorensis*” as a second flagship fish species in the school curricula in all stages. In which, the conservation story and the life history traits can be provided at different levels. The aim of this integration is to promote awareness and appreciation of the species and its conservation efforts among the young generations, who can provide more support in any future conservation programmes.

6. Increased regulation and risk assessment of non-native fishes in aquaculture.

The use of non-native fishes in aquaculture, such as *C. carpio* and *Oreochromis* spp. is likely to increase in future in order to maintain food security. The production within cages within newly created lentic waters created by impoundments is likely to occur. As such, regulators and water managers must work along aquaculturists to ensure high biosecurity to prevent escapes and utilise non-native risk assessments to ensure that the species that are used are those that only have a low risk of invasion should they escape into the wider environment. A joint awareness programme is recommended to the stakeholders who are engaged in water impoundment and distribution because they do not mind [in general] the introduction, and though it is of good benefit for the surrounding communities and water quality itself as a bio cleaning effects, where the invasion risk is out of the context.

7. Increase understandings of the interaction of climate change on the physical habitats of wadis, and the potential consequences for *G. ghorensis*; and

8. Complete further research on *G. ghorensis* populations, with emphasis on collating more precise data on specific aspects of their life cycle, and on crucial life stages.

The results of the research of this thesis have provided a great deal of information on *G. ghorensis*. However, to provide elevated understandings of the environmental and ecological needs of the species would require further research on the macro- and micro-habitat requirements of the species across all stages of their life cycle. At present, there remains a paucity of data on their more precise river flow requirements and little or no knowledge on the interaction of river flow with spawning and recruitment success. For the ecology of the species to strongly inform planning processes will require further and more precise work on specific aspects of the different life stages of *G. ghorensis*. These data can then be used within predictive models to develop greater understandings of how climate change and habitat disturbances could impact their population sustainability.

It is recommended to expand the research to include the effects of water flow on the life history traits using fixed flow loggers in the study sites. In addition, population size and population structure can be used as a precise indicator of the rivers health by measuring the length of living fishes and return them back without specimens removal. Where it is difficult to obtain population estimates, a proxy could be used such as catch per unit effort, where the number of fish captured during a standard time using standardized gears (e.g. gill nets or electric fishing) is expressed as the number of fish per unit time and unit effort (Harley et al., 2001). A baseline survey can set up the initial level of population size and structure that can be monitored every 3 years. A food availability studies could be invaluable, and seasonal food analysis is also recommended.

Finally, new modern techniques can be applied especially tagging either with microchips or more much advanced Platform Transmitter Terminal (PTT) radio tags or satellite tags that enable a wider understanding of fish movement patterns and associated life history traits in the disturbed environments.

9. Development of long-term *G. ghorensis* captive breeding programmes

Should recommendations 1 to 8 either be implemented and be unsuccessful, or overlooked and never implemented, then to also safeguard the species in the longer-term, some consideration should be given to creating a captive rearing programme. Although global experiences with captive rearing of fishes for subsequent release into the wild suggest there are inherent issues with rearing fishes that are artificially selected for their traits due to the culture process, i.e. released fishes have not be naturally selected, this activity would at least ensure that the species have the capacity to be re-introduced into waters where their populations have been extirpated due to habitat changes (Araki, et al., 2007, McPhee, 2004, Snyder, et al., 1996). However, these releases would only be completed once work had determined the fish were able to

survive and potentially develop sustainable populations in these habitats once more, such as following restoration work.

8.7 Conclusions

In conclusion, this research was successful in identifying the biogeographic origins of *G. ghorensis* and highlighted where historical geological and chemical changes had influenced their natural range in contemporary times. Surveys completed in 2010 revealed that despite continued alterations to the physical habitats of rivers within this range, this had yet to impinge on their distribution in the last decade. Studies on life history traits indicated *G. ghorensis* has some inherent phenotypic plasticity that enables them to adapt to some changes in their environment, with their relatively general diet and high reliance on algae and detritus enabling them to share resources with other fishes, both native and invasive, without necessarily having to compete. However, given their restricted natural range and constrained distribution within this, it is recommended that their status on the IUCN Red List as endangered remains, with a series of conservation and recommendations provided in Section 8.5 and 8.6 to help ensure the species do not undergo any population extirpations. Nevertheless, the outputs of Chapters 5 to 7 do indicate that some level of hydrological disturbances might be permissible across this range, but only where there is sufficient habitat remaining to ensure their populations still have all of their critical habitats remaining to complete their lifecycles. However, given the on-going and future issues of water security in the region that might be reduced by climate change, then for this to be successful is likely to require increased conservation efforts, including education programmes for key stakeholders.

9. References

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