Pertanika J. Trop. Agric. Sci. 38 (4): 413 - 426 (2015)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Invited Review Article

Current and Future Challenges of Conserving Freshwater Biodiversity: A Molecular Perspective

Jane M. Hughes

Australian Rivers Institute, Griffith University, Nathan, Queensland 4111, Australia

ABSTRACT

As the world's population continues to grow, human water needs are growing accordingly, thus reducing the water available for sustaining our freshwater biodiversity. This is likely to be further exacerbated in areas where rainfall will decrease as a result of global climate change. Molecular ecologists have contributed substantially in recent years to our understanding of first, the levels and patterns of current biodiversity and second, to understanding patterns of connectivity among populations of aquatic species and their significance for their conservation and management. Both are critical for prioritisation of areas for protection and for designing rehabilitation programmes. In this paper, I attempt to synthesise our understandings to date. I argue that a multi-disciplinary approach that incorporates new technological approaches in acquisition of molecular data is the best way forward for our aquatic biodiversity. Molecular ecologists can contribute by collaborating with other ecologists, especially in the fields of species distribution modelling and conservation planning. This approach will help to prioritise conservation actions for the best possible outcomes.

Keywords: Freshwater, Aquatic biodiversity, Climate change, Aquatic species, Molecular ecologists, Biological connectivity.

ARTICLE INFO

Article history:
Received: 25 June 2015
Accepted: 5 October 2015

E-mail address:
Jane.Hughes@griffith.edu.au (Jane M. Hughes)
* Corresponding author

INTRODUCTION

As the world's population continues to grow, human water needs are growing accordingly, thus reducing the water available for sustaining our freshwater biodiversity. This is likely to be further exacerbated in areas where rainfall will decrease as a result of

ISSN: 1511-3701

global climate change. Already, declines in freshwater biodiversity are far greater than in terrestrial systems (Sala, 2000; Strayer & Dudgeon, 2010). The major ways in which growing human demand for water will affect biodiversity include; increasing the number of dams, which already number over one million globally (Nilsson *et al*., 2005), and extraction of water for agriculture and aquaculture. These changes will have an array of negative impacts on biodiversity, but a major one is the impact on hydrological connectivity, which in turn will affect biological connectivity. Here, I use the term 'biological connectivity' to mean the connectivity between populations of a species, leading to gene flow, and also the connectivity between different parts of the habitat such as between rivers and floodplains, or between freshwater and marine conditions in the case of diadromous species. Connectivity can also refer to the flow of carbon and nutrients through the food web, but this form of connectivity will not be the focus of this review.

In order for competing needs to be managed, it is imperative that: first, we have accurate and efficient ways to assess our biodiversity. Currently, it is evident that species are going extinct more quickly than we can recognise them (Dudgeon *et al*., 2010). Second, we need accurate and efficient ways of assessing the current and historical connectivity among populations (as this will inform us of the potential for recolonisation following any local extinction – and also the degree of unique genetic diversity in populations that may represent adaptation to those environments). We also need to be able to assess the need for freshwater species to maintain connectivity with other habitats such as the floodplain or the estuary. Finally, given the competing needs of humans and aquatic biodiversity, we need to develop methods for prioritising which rivers, streams or reaches should best be preserved or protected in order to maximise protection of biodiversity. Headwaters are often less impacted by human influences than lower sections, but lower sections of rivers need to be maintained to sustain connectivity for migratory species (Pringle, 2001).

In this paper, I will:

1. Discuss recent advances in the assessment of biodiversity, the significance of cryptic species and how new information might affect prioritisation of regions for conservation.
2. Briefly discuss methods for assessing connectivity among intra-specific populations and determine if it might be possible to make generalisations about population connectivity based on a species' life-history and the habitat in which it lives (stream architecture).
3. Suggest some ways in which molecular approaches can be combined with other methods to understand migration patterns.
4. Finally, I will suggest future options that could improve our ability to conserve our freshwater biodiversity.

THE ROLE OF MOLECULAR APPROACHES IN ASSESSING BIODIVERSITY

Historically, aquatic biodiversity has been assessed using morphological information only (Jackson *et al*., 2014). While this has been the accepted method for many stream monitoring programmes, it has a number of drawbacks. One of the major ones is that there are few taxonomists sufficiently trained to be able to accurately identify many groups of invertebrates to species, especially as many invertebrates can only be identified as adults, but are usually collected as larvae from aquatic habitats. Furthermore, fewer taxonomists are being trained than in the past, so these skills are disappearing among our scientific community. For this reason, many programmes that monitor freshwater diversity identify organisms only to the family level (e.g., AUSRIVAS) (Smith *et al*., 1999). This clearly will be missing much of the diversity that exists below this taxonomic level and is likely to give a very inaccurate picture of the patterns of diversity across a landscape. The second problem is that there is a wealth of 'cryptic diversity' within freshwater taxa (Bickford *et al*., 2006; Jackson *et al*., 2014). By 'cryptic diversity', I mean species that are morphologically very similar, or even identical, but which are 'real species' in the sense that they have different geographic distributions, potentially different physiological tolerances, and in many cases, have been shown not to interbreed in nature (Baker *et al*., 2003; Cook *et al*., 2007). These 'cryptic species' are often identified initially from mitochondrial sequence data that are usually from the cytochorome oxidase 1 gene; the 'bar-coding gene' (Hebert *et al*., 2003). Having identified significant divergence in this gene, it is often possible for taxonomists to find distinguishing morphological characters, which before were just confusing, especially if multiple species occurred together, as for example in the genus *Paratya* in eastern Australia (Cook *et al*., 2006).

As more mitochondrial DNA work is amassed, large numbers of previously cryptic species are being recognised (Baker *et al*., 2004; Balint *et al*., 2011; Jackson *et al*., 2014). For example, *Caridina indisintcta*, described by Riek (1953) as a single species, has been shown to consist of a number of highly divergent lineages based on mtDNA. These lineages often co-occur in the same stream, making it difficult for taxonomists to determine whether they are looking at a single highly variable species or two or more coexisting species (Williams, 1977; Page *et al*., 2005). Allozyme analysis was used to demonstrate that the lineages did not interbreed where they co-occurred, as there were different alleles fixed in each lineage and no heterozygotes were detected at these loci (Woolschot *et al*., 1999). Furthermore, when a taxonomist examined species that had been identified using DNA, he was later able to describe morphological features that distinguished them (Page *et al*., 2005).

Many managers appear to think that geneticists are just describing additional intra-specific genetic variation when they talk about cryptic species, and that this

only represents differentiation between recently separated populations. There is a growing body of evidence, however, that many cryptic species are actually very 'old' (Bickford *et al*., 2006). Their morphologies may have remained unchanged due to unchanged selection pressures. In some cases, the features that differentiate species may not be visible in the form of different morphologies, but may, for example, involve discrete mating calls, pheromones, etc. One clear example of the importance of recognising cryptic species is the *Anopheles gambiae* species complex in Africa. There are seven cryptic species that vary in host preference and habitat – some only attack non-human hosts, thus posing no threat to humans. Recognition of these species allows management for human diseases to be focused only on those species that can impact humans (Besansky, 1999).

Another major issue with not recognising cryptic species is that real geographic distributions of the individual species are almost certainly a subset of the distribution of the single described morpho-species. Such is the case for the cryptic species of the freshwater mussel *Velesunio ambiguus* in eastern Australia, with two of the cryptic species being very widespread, while the other two have very limited distributions (Baker *et al*., 2002; Fawcett, 2008). The atyid shrimp *Paratya australiensis* consists of a complicated complex of cryptic species, with three species widespread across coastal and inland rivers systems, while other species are restricted to only a single or two nearby rivers (Figure 1) (Cook *et al*., 2006). Stuart *et al*. (2006) showed that 14 cryptic species could be recognised within two described species of the frog *Odorrana livida and Rana chalconota* in Southeast Asia. Each of the described species had a broad distribution, but the individual species distributions were much more limited, making them more prone to extinction, especially if managers were only working to protect certain populations of the nominal species. These authors were so far as to suggest that no widespread forest-dwelling frog species may occur in the region.

According to Dudgeon *et al*. (2006), extinction rates of freshwater animals in North America, based on data for unionid mussels, crayfishes, fishes and amphibians, are 4% per year, which is five times higher than the terrestrial average. This value could be much higher; However, this value could be much higher if cryptic species were taken into account, it is likely that many such species will go extinct as a result of human impacts even before they have been recognised formally.

Species distribution modelling uses a set of distribution records of a species, combined with a set of environmental variables to first, model the current distribution of each species. It then uses historical environmental records or future environmental predictions to predict past and future distributions. A study by Balint *et al*. (2011) sequenced mtDNA from nine aquatic insect species that inhabit alpine and subalpine regions and that are restricted to high altitude habitats because they are not tolerant of elevated ambient temperatures.

Within the nine formally described species, they identified potentially an additional 14 species. Using species distribution modelling, they predicted that three of the nine described species would go extinct by 2080, while 15 of the total 23 species would go extinct (Balint *et al*., 2011). This exemplifies the issue for aquatic diversity – as we have such poor assessments of it, knowing how to conserve and protect it is very difficult. These issues are exacerbated in tropical regions, where even less is known of the diversity (Dudgeon *et al*., 2006).

Conservation planning often uses outputs from species distribution modelling to prioritise the best areas to protect, given competing needs. However, conservation planning techniques have rarely been applied to cryptic species. This particular technique has been applied to freshwater fish species across northern Australia. They have assessed how regions that would be given priority would change depending on the taxonomic level at which the analysis was done. They used conservation planning tools to determine the areas that would be required to conserve 500 Km^2 of riverine habitat for each taxon. They did this in three ways, at the genus level, the described species level and the cryptic species level (identified using molecular data). There were 43 genera, 87 described species and a total of 143 species when molecular data were incorporated. They found that if the prioritisation were done at the genus level, only 60% of the total number of species (i.e., all the species identified using molecular data) would be conserved, and 1.5% of the total area would be required. If this was done at the described species level, still only 70% of the actual species would be preserved, and 2.5% of the total area would be required. By basing the analysis on all species, obviously all would be conserved, and this would require only an additional 1% of the total area. This suggests that conservation planners should take into account information on cryptic species, where it is available.

CONNECTIVITY AMONG POPULATIONS

It is extremely important to understand the way in which populations are connected in order to manage them effectively. Populations that are strongly connected with other populations are unlikely to suffer from chance local extinctions for long, because they will be quickly recolonised. In contrast, populations that are isolated from other populations of the same species are likely to suffer from reduced genetic diversity, as they will lose diversity through random genetic drift and if they do go extinct, recolonisation is much less likely. Over the last 20-30 years, ecologists have applied a range of techniques to understand dispersal among populations of freshwater species. Early studies used allozymes (Schmidt *et al*., 1995), while later studies used mitochondrial DNA sequence data (Schultheis *et al*., 2002) and more recently microsatellite data (Hughes *et al*., 2014). All these approaches rely on the very simple idea that if there is dispersal and gene flow between two populations, then they will tend

to contain a similar genetic composition, whereas if gene flow is limited between populations, gene frequencies will tend to diverge as a result of genetic drift and or selection (Slatkin, 1985). The degree to which populations are connected by gene flow and hence the genetic similarities among them has been empirically shown to be affected by a number of life-history factors and the habitats in which they live. For example, genetic similarity between populations has been shown to be greater for species with a flight stage in their life-history than for those that are solely restricted to the stream (Bohonak & Jenkins, 2003; Hughes, 2007) and species that occur in upstream sites tend to be more genetically differentiated than those that occur in lowland habitats (Hughes, 2007). In fact, Finn *et al*. (2011) demonstrated that for a group of freshwater insects and crustaceans, headwaters contained higher differentiation among sites than even populations in streams only slightly further down the stream hierarchy. If this finding is generally the case, then protecting headwater streams becomes even more important.

Not only is it important to know how much dispersal and gene flow occurs between populations, but it is also important to know the patterns of connectivity among populations. A number of models have been proposed to describe the way in which populations of riverine species are connected. The stream hierarchy model (Meffe & Vrijenhoek, 1988) proposes that riverine species should show patterns of connectivity that reflect the dendritic structure of the stream network. The highest levels of connectivity should be among sites within a stream, with connectivity decreasing with level in the hierarchy, and the lowest between sub-catchments or catchments. Such a model was originally suggested to apply to obligate freshwater fish, but also has been shown to apply to many other riverine species (e.g., crustaceans: Bunn & Hughes, 1997; stoneflies: Hughes *et al.*, 1999). The death valley model (Meffe & Vrijenhoek, 1999) was proposed for species that live in isolated waterholes that are rarely connected by surface flows. All populations are highly differentiated and there is no relationship between connectivity and position in a catchment network, nor with geographic distance separating them. The headwater model (Finn *et al*., 2007) was proposed for headwater specialists that have some abilities to move out of the stream. If their streams confluence outside their tolerance limits (i.e., at low altitudes), then connectivity is likely to be higher across the catchment divide than within the same catchment. The final model is panmixia, which describes systems where gene flow is very widespread and there is no relationship between connectivity and position in the catchment, nor with geographic distance. This model applied to some aquatic insects with strong-flying adults such as dragonflies and dytiscid beetles (Phillipsen *et al*., 2015).

Knowledge of the model that a species fits in terms of patterns of biological connectivity can assist managers in a number of ways. For example, if a species fits the stream hierarchy model, the outcomes of

different disturbance events can be predicted more successfully. If populations in a whole subcatchment are extirpated by, for example, a pollution event, then natural recolonisation is much less likely than if the same number of populations is removed patchily across the stream network, as might happen during a drought. If a species is effectively panmictic throughout the system, then local extinctions will have little impact, as they will quickly be recolonised from other parts of the system. Local extinctions in populations of headwater species within a sub-catchment will only be recolonised if there are potential source populations on the other side of the drainage divide. Species that fit the death valley model are likely to contain populations that are significantly diverged from one another, may be adapted to local conditions, and local extinctions are unlikely to be recolonised. Furthermore, translocation of individuals among these populations and transferring water between basins is likely to present risks to endemic populations such as extinctions of both types (Hughes *et al*., 2003), and introgression and loss of adaptive potential (Allendorf *et al*., 2001).

Hughes *et al.* (2013) proposed that it should be possible to predict the model that a given species should fit based on knowledge of the species life-history and the stream architecture/geography of its habitat. They developed a decision tree approach to assist managers with predicting patterns of connectivity. They tested this idea with data from 47 fish studies and 62 invertebrate studies. Predictions were correct for more than 70% of both fish and invertebrate cases, but there were still enough species that did not fit the predictions to suggest that data on individual species are still the best way to do this. Nevertheless, where there is no datum available for a given species, at least this approach may give managers a place to start.

ASSESSING CONNECTIVITY FOR SPECIES THAT MOVE BETWEEN HABITATS AS PART OF THEIR LIFE-HISTORY

Many species move quite large distances as part of their life-history. Possibly the most extreme of these are the diadromous species that spend part of their lives in fresh water and part in marine conditions. There are three main forms of diadromy: anadromy (where reproduction occurs in freshwater), then juveniles migrate to the sea and spend most of their lives in marine conditions before returning to the freshwater to breed, catadromy (where individuals go to the ocean to breed), but then return to freshwater as juveniles, where they spend most of their lives, and amphidromy, migration between marine and freshwaters habitats but not for the purpose of reproduction (Myers, 1949). Until recently, understanding these behaviours has been proven difficult for ecologists because it requires tagging large numbers of individuals that are likely never recaptured. Genetic markers are not useful because they can only give information concerning the eventual outcomes of successful reproduction.

In fish, this problem has been partly solved by using information contained in the fish otolith (or earbone). These structures are

composed of calcium carbonate and are laid down in a series of rings as the fish grows. While they are predominantly calcium carbonate, they also take up tiny amounts of other elements from the medium in which they occur (Hicks *et al*., 2100). The otolith thus contains a permanent record of where the fish has been residing. By combining the fact that the rings give an estimate of age with the fact that the composition will reflect where the fish was when that ring was laid down, it is possible to infer the history of an individual fish by running a transect through the otolith and analysing particular elements. This can be done using laser ablation mass spectrometry (Hughes *et al*., 2014). For examining diadromy, stable isotopes of Strontium have been shown to be particularly useful. Sea water has a very consistent ratio of ^{86}Sr to ^{87}Sr, while freshwater systems have ratios that reflect the geology of the surrounding catchments, so they often differ significantly from seawater and from one another. Hughes *et al*. (2014) studied a small amphidromous fish, which had been predicted to have little genetic variation across its range due to the fact that eggs and larvae were thought to go to the ocean and were thus likely to be mixed by ocean currents. Using Sr isotope ratios, they were able to show that some populations were composed totally of diadromous individuals, some populations were totally freshwater – and a small number contained a mixture of both. Even more surprisingly for an amphidromous species, populations in different river systems were highly genetically differentiated from one another. Further use of otolith chemistry showed that this was probably because although many populations spent time in marine conditions, this time was spent in individual estuaries rather than in the open ocean. This was inferred by multi-elemental analysis, which showed that populations differed in the core of their otoliths, a result that would not have been expected if they had come from the relatively homogeneous open ocean.

Studying within life-cycle migration is more difficult for invertebrates, as they do not possess otoliths. Some studies have used stable isotope signatures of soft body parts (Cook *et al*., 2007), which may show differences among streams and between marine and freshwater and hence aid in understanding migration. The drawback with using soft tissues, however, is that the signature only remains for a limited time, a period of weeks to months (Fraser *et al*., 1997). New work on hard parts of crustaceans such as eye-stalk and gastric mill has suggested that they retain signatures that assist with age determination (Kilada *et al*., 2012). These structures are also composed of calcium carbonate, so could presumably be used in the same way as otoliths to study migration patterns. So far, no one appears to have done this.

FUTURE CHALLENGES AND POSSIBILITIES

Deciding which catchments, rivers or stream reaches should be prioritised for

protection in the future will be a daunting task. However, some new technologies may make this slightly easier.

One possible answer to the taxonomic dilemma has come from recent advances in DNA sequencing. Next Generation Sequencing (NGS) allows the sequencing of many individuals in a single run. While the cost of these techniques was initially out of the range of most stream ecologists, prices are reducing all the time. It is possible, using the DNA bar-coding gene to analyse samples of sediment or water and to obtain mtDNA sequences from all (or at least many) of the organisms present in the sample (Shokralla *et al*., 2012; Thomsen *et al*., 2012). Depending on the technology used, more than single samples can be analysed in a single run. While the repeatability and feasibility of these approaches are still being determined, it is clearly a way of the future. The sequences obtained from the run can be combined with others of known described species from Genbank and put into a phylogenetic tree. This information can then be used to identify species in the sample – or even to identify sequences that may belong to new undescribed species. By using these techniques, ecologists should be able to obtain a reasonable idea of the species present at their site. There are still some problems with interpretation of these data though. The first is that at present, using these techniques to determine abundance of particular species may be problematic, for a few reasons. The first is that it is likely that more DNA sequences will be obtained from larger species. The second is that the polymerase chain reaction (PCR), which is used to amplify DNA fragments from the initial mixed sample, may preferentially amplify some species over others. Even so, this technique holds great promise for assessing biodiversity in the future, as demonstrated already for chironomids (Carew *et al*., 2013). These approaches are also likely to be useful for detecting the presence of threatened species that are difficult to catch and also to provide early warning of the presence of invasive species.

As we amass more information about the levels and patterns of connectivity among populations of aquatic species in different environmental settings and with different life-histories, especially by combining a range of approaches, we should be able to improve our generalisations about how best to manage them. For example, for amphidromous species that require access to the marine environment in order for larvae and eggs to develop, efforts should be made to maintain hydrological connectivity between river and ocean. Currently, this can be blocked in a number of ways: by building dams on the rivers so that larvae cannot get access to the estuary; secondly, if there is major pollution in downstream reaches that affects larvae, this could also restrict migration. Finally, in many high wave energy coasts such as in southern Australia, connections to the sea are intermittent. If flows are low, the waves deposit sand at the mouth and build bars, which stop the rivers from reaching the sea. As rainfall levels drop, as is predicted for southern Australia, and if significant water

is abstracted from the rivers along their way, then they might not open for a number of years. This will result in local extinctions of many diadromous species. Whether or not they can recover will depend on whether there are nearby source populations that can recolonise. This is not just an issue for southern Australia. Several of the world's large rivers (Ganges, Nile, Colorado) have already stopped flowing to the sea during prolonged dry periods (Strayer & Dudgeon 2010).

With more and more information available from DNA, both traditional mtDNA sequence data and those obtained from NGS, we are likely to identify many new species or at least new lineages that have apparently been isolated from the rest of their species for millions of years. Where possible, this information should be incorporated into conservation planning approaches, so that at least the cryptic diversity can be taken into account.

While currently conservation planners attempt to identify regions of high diversity and endemism based on described species, knowledge of the distributions of cryptic species may well identify different areas as being important. It will be very useful in the near future to consolidate all the information appearing on cryptic species to produce more meta-analyses that incorporate this. One approach may be to use what knowledge we have on the distribution of molecular diversity across sub-catchments and catchments to define molecular bioregions.

Finally, it appears that the freshwater biodiversity of tropical systems is particularly poorly known and should be given priority, possibly by setting up large collaborations across the world. Potentially, the DNA bar-coding project should contribute to this.

ACKNOWLEDGEMENTS

This manuscript was modified from a plenary address given to the Society for Freshwater Sciences in Milwaukee in May, 2015. Peter Mather commented on an earlier draft of the manuscript.

REFERENCES

Allendorf, F. W., Leary, R.F., Spruell, P., & Wenburg, J. (2001). The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution, 11*, 613-622.

Baker, A. M., Hughes, J. M., Dean, J. C., & Bunn, S. E. (2004). Mitochondrial DNA reveals phylogenetic structuring and cryptic diversity in Australian macroinvertebrate assemblages. *Marine and Freshwater Research, 55*, 629-640.

Baker, A. M., Bartlett, C., Bunn, S. E., Goudkamp, K., Sheldon, F., & Hughes, J. M. (2003). Cryptic species and morphological plasticity in long-lived bivalves (Unionoida: Hyriidae) from inland Australia. *Molecular Ecology, 10*, 2707-2717.

Balint, M., Domisch, S., Engelhardt, C. H. M., Haase, P., Lehrian, S., Sauer, J., Theissinger, K., Pauls S. U., & Nowak, C. (2011). Cryptic biodiversity loss linked to global climate change. *Nature Climate Change, 1*, 313-318.

Besansky, N. J. (1999). Complexities in the analysis of cryptic taxa within the genus *Anopheles*. *Parasitologia, 41*, 97-100.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., &

Das, I. (2006). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution, 22*, 148-155.

Bohonak, A. J., & Jenkins, D. G. (2003). Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters, 6*, 783-796.

Bunn, S. E., & Hughes, J. M. (1997). Dispersal and recruitment in streams: evidence from genetic studies. *Journal of the north American Benthological Society, 16*, 338-346.

Carew, M. E., Pettigrove, V. J., Metzling, L., & Hoffman A. A. (2013). Environmental monitoring using next generation sequencing: rapid identification of macroinvertebraet bioindicator species. *Frontiers in Zoology, 10*, 45.

Cook, B. D., Baker, A. M., Page, T. J., Grant, S. C., Fawcett, J. H., Hurwood, D. A., & Hughes, J. M. (2006). Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): the role of life-history transition in phylogeographic diversification. *Molecular Ecology, 15*, 1083-1093.

Cook, B. D., Bunn, S. E., & Hughes, J. M. (2007). A comparative analysis of population structuring and genetic diversity in sympatric lineages of freshwater shrimp (Atyidae: *Paratya*): concerted or independent responses to hydrographic factors. *Freshwater Biology, 52*, 2156-2171.

Cook, B. D., Bunn, S. E., & Hughes, J. M. (2007). Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable southern pygmy perch (*Nannoperca australis*). *Biological Conservation, 138*, 60-72.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z-I., Knowler, D. F., Leveque, C., Naiman, R. J., Prieu-Richard, A-H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biology Reviews, 81*, 163-182.

Elith, J., & Leathwick, J. R. (2009). Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology and Systematics*, 677-697.

Fawcett, J. H. (2008). *Muddy waters: a molecular approach to clarifying freshwater mussel diversity in Australia*. (PhD thesis dissertation). Griffith University, Brisbane.

Finn, D. S., Blouin, M. S., & Lytle D. A. (2007). Population genetic structure reveals terrestrial affinities for a headwater stream insect. *Freshwater Biology, 52*, 1881-1897.

Finn, D.S., Bonada, N., Murria, C., & Hughes, J. M. (2011). Small but mighty: headwaters are vital to stream network diversity at two levels of organization. *Journal of the North American Benthological Society, 30*, 963-980.

Fraser, T. K., Ross, R. M., Quetin, I. B., & Monteur, J. P. (1997). Turnover of carbon and nitrogen during growth of larval krill *Euphausia superba* Dana: a stable isotope approach. *Journal of Experimental Marine Biology and Ecology, 212*, 259-275.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identification through DNA barcodes. *Proceedings of the Royal Society B- Biological Sciences, 270*, 313-321.

Hughes, J. M., Mather, P. B., Sheldon, A. L., & Allendorf, F. W. (1999). Genetic structure of the stonefly *Yoraperla brevis*: the extent of gene flow among adjacent montane streams. *Freshwater Biology, 41*, 63-72.

Hughes, J. M., Goudkamp, K., Hurwood, D. A., Hancock, M., & Bunn, S. E. (2003). Translocation causes extinction of a local population of the freshwater shrimp *Paratya australinesis*. *Conservation Biology, 17*, 1007-1012.

Hughes, J. M., Schmidt, D. J., McDonald, J. I., Huey, J. A., & Crook, D. A. (2014). Low inter-basin connectivity n a facultatively diadromous fish: evidence form genetics and otoliths. *Molecular Ecology, 23*, 1000-1013.

Jackson, J. K., Battle, J. M., White, B. P., Pilgrim, E. M., Stein, E. D., Miller, P. E., & Sweeney, B. W. (2014). Cryptic biodiversity in streams: a comparison of macroinvertebrate communities based on morphological and DNA barcode identifications. *Freshwater Science, 33*, 312-324.

Kilada, R., Bernard, S-M., & Remy, R. (2012). Direct determination of age in shrimps, crabs and lobsters. *Canadian Journal of Fisheries and Aquatic Sciences, 69*, 1728-1733.

Meffe, G. K., and Vrijenhoek, R. C. (1988). Conservation genetics in the management of desert fishes. *Conservation Biology, 2*, 157-169.

Myers, G. S. (1949). Usage of anadromous, catadromous and allied terms for migratory fishes. *Copeia, 2*, 89-97.

Page, T. J., Chooy, S. C., & Hughes, J. M. (2005). The taxonomic feedback loop: symbiosis of morphology and molecules. *Biology Letters, 1*, 139-142.

Phillipsen, I. C., Kirk, E. H., Bogan, M. T., Mims, M. C., Olden, J. D., & Lytle, D. A. (2015). Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. *Molecular Ecology, 24*, 54-69.

Pringle, C. (2001). Hydrologic connectivity and the management of biological reserves: a global perspective. *Ecological Applications, 11*, 981-998.

Riek, E. F. (1953). The Australian freshwater prawns of the family Atyidae. *Records of the Australian Museum, 15*, 11-121.

Schmidt, S. K., Hughes J. M., & Benn, S. E. (1995). Gene flow among conspecific populations of *Baetis* sp. (Ephemeroptera)- adult flight and larval drift. *Journal of the North American Benthological Society, 14*, 147-157.

Schultheis, A. S., Weight, L. A., & Hendricks, A. C. (2002). Gene flow, dispersal and nested clade analysis among populations of the stonefly *Peltoperla tarteri* in the southern Appalachians. *Molecular Ecology, 11*, 317-327.

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology, 21*, 1794-1805.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and Systematics, 16*, 393-430.

Smith, M. J., & Williams, W. D. (1980). Intraspecific variation within the Atyidae: a study of morphological variationwithin a population of *Paratya australiensis* (Crustacea: Decapoda). *Australian Journal of Marine and Freshwater Research, 31*, 397–407.

Smith, M. J., Kay, W. R., Edward, D. H. D., Papas, P. J., Richardson, K. S., Simpson, J. C. Pinder, A. M. Cale, D.J., Horwitz, P. H. J., & Davis, J. A. (1999). AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. *Freshwater Biology, 41*, 269-282.

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society, 29*, 344-358.

Stuart, B. L., Inger, R. F., & Voris, H. K. (2006). High levels of cryptic diversity revealed by sympatric lineages of Southeast Asian forest frogs. *Biology Letters, 2*, 470-474.

Thomsen, P. F., Kilgast, J., Iversen, L. L., Wiuf, C., Rasmussen, F., Gilbert, M. T. P., Orlando, L., & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology, 21*, 2565-2573.

Willaims, W. D. (1977). Some aspects of the acology of *Paratya australiensis* (Crustacea Decapoda Atyidae). *Australian Journal of Marine and Freshwater Research, 28*, 403-415.

Woolschot, L., Hughes, J. M., & Bunn, S. E. (1999). Dispersal among populations of *Caridina* sp. (Decapoda: Atyidae) in coastal lowland streams, south-east Queensland Australia. *Australian Journal of Marine and Freshwater Research, 50*, 681-688.