Conservation genetics as a tool for conservation and management of the native Japanese freshwater crayfish *Cambaroides japonicus* (De Haan)

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**Abstract.**—Unprecedented rates of extinction have lead to the development of fields, such as conservation genetics, in an attempt to better understand biodiversity and consequentially devise conservation programs to maintain the genetic integrity of species. We discuss the utility and application of conservation genetics, using examples from Australian freshwater crayfish, with a view to applying this methodology to the sole native crayfish species found in Japan, *Cambaroides japonicus* (De Haan). This species is threatened by the non–indigenous crayfish *Pacifastacus leniusculus* (Dana) through displacement, competition pressures, and susceptibility to *Aphanomyces astaci* Schikora (the crayfish plague, carried by the invasive species). Examining the genetic diversity within *C. japonicus* could allow populations of high conservation priority to be identified (i.e., genetically distinct populations) or, in contrast, show that this species is genetically homogenous. Such genetic continuity may suggest that other conservation methods (e.g., translocations and restocking populations) may be suitable for this species of crayfish. Identifying genetic variability, or lack thereof, is a key step in dictating the future direction of any conservation measures for *C. japonicus*.

**INTRODUCTION**

It is apparent that throughout the world, population declines and species’ extinctions are occurring at an unprecedented rate (Dirzo & Raven, 2003; DeSalle & Amato, 2004; May, 2010). Although extinctions are natural phenomena, evidence suggests that current extinction rates far exceed those seen in the past and are likely, for the most part, attributable to human actions (World Conservation Monitoring Centre, 1992). While it has been established that terrestrial, marine, and freshwater ecosystems have all been affected, extinction rates are difficult to accurately estimate due to our incomplete understanding of current and past levels of biodiversity. Nevertheless, previous researchers have identified that biodiversity within freshwater ecosystems is at particular risk (e.g., Saunders, 1976; Dudgeon, 1984; Sala et al., 2000; Cook et al., 2008); although this environment is relatively understudied in comparison to terrestrial and marine systems.

Recent studies employing molecular techniques have revealed cryptic diversity in formerly well–studied groups of organisms (Baker et al., 2004), and cryptic genetic divergence in the world’s largest and arguably most iconic freshwater crayfish species *Astacopsis gouldi* Clark (Sinclair et al., 2011). These studies highlighted both our incomplete understanding of well–known groups and species, but also that very high group– and species–level conservation values can be easily overlooked.

This review is concerned with the status of one particular group of aquatic organisms, freshwater crayfish, which are found on every continent (excluding the African (bar Madagascar), Antarctic, and Indian sub–continent) and are widely regarded as keystone species (*sensu* Krebs, 1994) in areas they inhabit (Hart, 1992; Momot, 1995; Holdich, 2002c).

In 2010, the world’s freshwater crayfish were assessed for the IUCN Red List of Threatened Species, and the evaluation of 528 species established that freshwater crayfish are among the world’s 5 most
threatened groups of animals (Dewhurst, 2010 personal communication; IUCN, 2011). As first highlighted by Wells et al. (1983), the main threats to invertebrates and freshwater crayfish are: 1) habitat destruction, 2) pollution, 3) non-indigenous species, and 4) human exploitation (Wells et al., 1983; IUCN, 2011). Recently identified emerging threats include changes to climate and increasing environmental temperature (Horwitz, 1990a; 2010; Coughran & Furse, 2010; Furse & Coughran, 2011a; Furse et al., 2012a) and increased intensity and frequency of severe weather events (Furse et al., 2012b).

The objective of this paper is to review the conservation status of the sole native Japanese species of freshwater crayfish (Cambaroides japonicus (De Haan)), and introduce and discuss the applications of existing techniques/methods that could assist in conservation efforts for this species. Examples of how these techniques have been applied in the context of Australian freshwater crayfish will be provided, with particular focus on determining levels of genetic diversity and how this data can be used for conservation assessments and recovery and management efforts.

Current Conservation Status of C. japonicus

Cambaroides japonicus has been assessed versus IUCN Red List criteria as Data Deficient (DD) with a declining population trend (Kawai & Machino, 2010). The DD assessment was primarily due to a lack of information regarding its distribution and extent of occurrence (EOO) defined by the IUCN as “the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon” (IUCN 2010). The current DD assessment for C. japonicus should not be interpreted as a stable or improving conservation status as it is known the species faces a number of very serious and ongoing threats (Nakata et al., 2002; Kawai & Machino, 2010).

For example, some iconic, and comparatively well-studied species are facing a suite of well known and serious threats, and are consequently of considerable conservation concern; yet they are currently classified as DD. The World’s 2nd largest species of freshwater crayfish, Euerastacus armatus (von Martens) from Australia is one such species; the species faces numerous serious threats, but a simple lack of quantified data on its contemporary distribution and abundance does not permit assessment versus IUCN Red List criteria (Furse & Coughran, 2011b; IUCN, 2011). The DD assessment for C. japonicus highlights the need for more basic research on its contemporary distribution and occupancy of habitat.

In their 2010 assessment, Kawai and Machino identified key threats to C. japonicus, the most serious of which was the spread of the non-native and highly invasive American signal crayfish, Pacifastacus leniusculus (Dana), which was introduced to the Japanese main Islands in 1928 and has been expanding its distribution since that time (Nakata & Goshima, 2003).

There is great deal of concern over the continued spread of this crayfish, including any human translocations, for a numbers of reasons: 1) the species is robust and can tolerate a wide range of environmental conditions, including near arctic (i.e., latitude 62°N, Heinimaa & Pursiainen, 2008), 2) P. leniusculus has superior competitive abilities over the smaller C. japonicus, and 3) P. leniusculus is a known ‘healthy carrier’ of Aphanomyces astaci Schikora (hereafter, crayfish plague) which is highly contagious and uniformly fatal to susceptible species (which includes C. japonicus) (Evans & Edgerton, 2002).

The invasive potential, competitive advantage, and capacity of P. leniusculus to act as a disease vector (i.e., to spread crayfish plague between waterbodies) are well documented and reasonably well understood in regions where the species has been introduced, spread, and been intensively studied. Additional threats to native species are via interspecific competition and interspecific breeding, resulting in reduced
reproductive success in native species (e.g., Söderbäck, 1991; 1994; Westman et al., 2002). The United Kingdom (UK) and parts of mainland Europe are good examples of where entire populations of native species have been extirpated, and where surviving native populations are still at threat from the invading species (Evans & Edgerton, 2002; Jussila et al., 2008; Bjurström et al., 2010; Peay et al., 2010).

Eight native crayfish species are recognised in Europe, and while not all have been assessed for the IUCN Red List (Table 1), the presence of invasive crayfish (e.g., P. leniusculus and Procambarus Clarkii (Girard)) and as such are considered as very serious threats to the native species (IUCN, 2011).

Europeans have a long history of social customs and traditions associated with crayfish (e.g., the annual Swedish “Crayfish Parties”), as well as industries such as wild fisheries and aquaculture (Holdich, 2002b; 2002a), and these traditions and industries are threatened by invasive species. As non-native species are reasonably resilient and robust, quite widely distributed, and typically such dominant competitors, it is considered unlikely that native species will be able to recover to pre-invasion levels (Holdich et al., 2009).

Examination of the situation in Japan reveals many similarities to Europe. Studies have compared the biology and ecology of the native C. japonicus and non-indigenous P. leniusculus and concluded that P. leniusculus is likely to out-compete C. japonicus for habitat and resources, owing to its higher fecundity (and generally higher reproductive capacity), higher growth rates, and resistance to diseases including the crayfish plague (Yamanaka et al., 1997; Nakata et al., 2004).

Direct predation and increased success in obtaining shelter/refuges (a limiting variable for presence and density of crayfish) and reduced refuge availability have also been indicated as threats to the survival prospects of C. japonicus (Nakata et al., 2006).

Research Imperatives and Methods for Investigation of C. japonicus

Addressing the previously documented knowledge gaps for C. japonicus should be accorded high priority (and allocated appropriate funding) as the resulting information will allow a thorough evaluation of the species’ conservation status, and any conservation and management measures that may be required. In particular, it is imperative that the geographical distribution and taxonomic uncertainties (i.e., any cryptic diversity) of C. japonicus are investigated and clarified.

In light of experience elsewhere in the world, genetic techniques can play a central role in facilitating the timely collection of information and subsequent conservation evaluations that are required for this species.

Genetic techniques have been used in a number of countries to assess factors such as

<table>
<thead>
<tr>
<th>Species and Authority</th>
<th>IUCN Red List Status and Population Trend</th>
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<tbody>
<tr>
<td>Austropotamobius pallipes (Lereboulet)</td>
<td>Endangered (EN), Trend: Decreasing</td>
</tr>
<tr>
<td>Astacus astacus (Linnaeus)</td>
<td>Vulnerable (VU), Trend: Decreasing</td>
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<tr>
<td>Astacus leptodactylus (Eschscoltzt)</td>
<td>Least Concern (LC), Trend: Unknown</td>
</tr>
<tr>
<td>Astropotamobius torrentium (Shrank)</td>
<td>DD, Trend: Decreasing</td>
</tr>
<tr>
<td>Astacus pachyplus (Rathke)</td>
<td>DD, Trend: Unknown</td>
</tr>
<tr>
<td>Austropotamobius italicus italicus (Faxon)</td>
<td>Not Assessed, Trend: Unknown</td>
</tr>
<tr>
<td>Austropotamobius italicus carsicus M. Karaman</td>
<td>Not Assessed, Trend: Unknown</td>
</tr>
<tr>
<td>Austropotamobius italicus carinthiatus Albrecht</td>
<td>Not Assessed, Trend: Unknown</td>
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</table>
Genetic diversity in Australian crayfish species

Examining genetic diversity within populations for the purpose of conservation is part of the relatively new field of conservation genetics. Conservation genetics combines genetic information with ecological and distributional information to identify geographic regions or localities where species are most at risk (O'Brien, 1994). Identifying geographical areas where unique and/or discrete genetic populations exist effectively prioritises these areas as ‘of conservation concern’. One common method for delineating such populations is by identifying them as evolutionarily significant units (ESUs, Moritz, 1994), using information obtained from both mitochondrial (mtDNA) and nuclear DNA (nuDNA) (as outlined in Moritz, 1994). In the Australian context, ESUs have been recognised in a variety of freshwater crayfish, both widespread and with highly restricted and/or fragmented distributions, and these are outlined in the following sections.

Cherax tenuimanus (Smith)

Cherax tenuimanus is endemic to Western Australia and is one of the world’s largest freshwater crayfish species: IUCN Status Critically Endangered (CR) (IUCN, 2011).

This is an important species for aquaculture (Imgrund et al., 1997) and for cultural reasons due to a large and well-established recreational fishery (Bunn et al., 2008). Due to extensive translocations and hybridisation (Bunn et al., 2008), the genetic integrity of this species has been raised as a serious conservation concern. Maintaining genetic diversity (and eliminating hybrids) is a management priority for both commercial and wild populations (see Bunn et al., 2008).

Nguyen et al. (2002), examined the genetic variation within the mtDNA gene region 16S between populations throughout Western Australia as well as translocated populations located in South Australia and Victoria. Two distinct genetic groups were identified (one from Margaret River in southern Western Australia, the other consisting of all other populations that were evaluated); it was recommended that both groups be recognised as ESUs, with the Margaret River ESU represented by only a single population. The extremely high conservation value of the Margaret River population (versus the other populations) was highlighted in this study, as it was “restricted to this single river system and represents the only significant genetic diversity within the species so far recorded” (Nguyen et al., 2002).

Without identification of this genetic diversity (i.e., distinct genetic structure), consequences for the Margaret River population could have been dire, including; translocations, potential cross-breeding, and quite possibly extirpation of the unique genetic stock in the population. The Nguyen et al. (2002) study also identified negative genetic outcomes such as introgression and outbreeding depression, which can lead to decreased population fitness.

Cherax destructor Clark

Cherax destructor has the most widespread natural geographic distribution in Australia and extends into the semi-arid zone: IUCN Status VU (IUCN, 2011).

Cherax destructor has a history of widespread and long-range translocations both pre- and post-European settlement (Horwitz, 1990b). These translocations are on-going (Coughran et al., 2009; Coughran & Daly, 2012) and are of considerable concern for conservation of native species. Horwitz & Knott (1995) suggested that
such extensive and expansive movements of *C. destructor* may have resulted in genetic homogenisation throughout the species. Examination of the mtDNA 16S gene region by Nguyen *et al.* (2004) found three major lineages within this species and consequently recommended them as ESUs. *Cherax destructor* populations are under considerable pressure from a large and widespread recreational fishery, aquaculture activities, and ongoing rural development (e.g., agriculture) (Austin *et al.*, 2003; Nguyen *et al.*, 2004). However, the close geographic proximity of the three discrete lineages is an additional concern for maintaining the genetic integrity of wild *C. destructor* populations (Austin *et al.*, 2003).

**Tenuibranchiurus glypticus Riek**

The sole species in this genus is the smallest freshwater crayfish species in Australia: IUCN status EN (IUCN, 2011).

Due to its very small size with a total length (rostrum – telson) < 35 mm, and highly fragmented distribution, this crayfish has effectively no dispersal capabilities (Coughran *et al.*, 2010; Dawkins *et al.*, 2010). The species is restricted to coastal *Melaleuca* swamp habitat along the central eastern coast of Australia, with habitat patchiness and biological restrictions (e.g., small size) thought to be the cause of the high levels of genetic divergence between isolated populations (Dawkins *et al.*, 2010).

Examination of both mtDNA (Dawkins *et al.*, 2010) and nuDNA (authors unpublished data) from the various geographically isolated populations show very high levels of genetic diversity, and suggests recognition of at least 5 ESUs is warranted based on mtDNA data (Dawkins *et al.*, 2010). Genetic data also indicates the presence of at least one additional previously unidentified species (Dawkins *et al.*, 2010). Such high levels of previously unknown genetic divergence highlight the utility of genetic methods/techniques as powerful conservation tools. In this particular case it has allowed a potential new species to be identified, and genetically unique populations to be considered and treated as extremely high conservation priorities. This is particularly important as coastal regions of Australia are becoming increasingly urbanised (ABS, 2009), threatening many of the remaining habitat fragments that these species occupy (Dawkins, 2007; Coughran *et al.*, 2008).

**Conservation implications of identifying cryptic diversity**

An example of the implications of identifying cryptic diversity in a reasonably well–known and widespread species is provided by the case of *Euastacus sulcatus* Riek (IUCN Status: VU). The species contains a number of small and geographically isolated populations, and Coughran & Furse (2010) and Furse & Coughran (2011b) identified the possibility that genetic analysis could identify a number of genetically distinct populations (or distinct species). If assessed versus IUCN criteria at the population level, as opposed to the current species–level assessment, some *E. sulcatus* populations would warrant listing as Critically Endangered or EN (Coughran & Furse 2010). There is potential for a similar situation to apply in the case of *C. japonicus* in Japan.

**Application of conservation genetics in Japan**

As the geographical range of *P. leniusculus* appears to be increasing in Japan, and, as no successful methods of eradicating this species are known, the primary focus of conservation efforts for *C. japonicus* should include identifying any populations that possess unique genetic information (i.e., cryptic diversity), identifying them as ESUs and assigning them critical conservation status.

By identifying any priority areas of critical habitat (i.e., areas containing ESUs), conservation management can first focus on those populations that are most ‘genetically valuable’ (and potentially saveable) and then efforts can be extended to remaining populations of lesser priority. Any “invasion–proof” areas of suitable *C. japonicus* habitat should also be identified. Isolated areas of habitat suitable for *C. japonicus* (i.e., without non–indigenous crayfish, and not threatened by invasion by non–indigenous crayfish)
that are able to be maintained in the long term could prove invaluable for conservation of this native Japanese species (sensu ‘Ark Sites’ in the UK) (Holdich et al., 2009).

If ESUs are not indicated and populations are identified as genetically homogenous, this also provides other avenues for conservation efforts. For instance, one of the primary concerns for genetically distinct populations (as previously outlined) is the potential for deleterious effects occurring through translocations. For example, if C. japonicus is genetically homogenous then it may be possible and feasible for areas experiencing population declines to be ‘restocked’ to mitigate any such reductions.

Furthermore, if successful eradication methods are developed for P. leniusculus, areas previously occupied by the native species could possibly be repopulated. Such methods would of course require careful consideration of other factors, including the potential of inadvertently translocating diseases and organisms such as commensals and parasites.

By simply examining the genetic diversity within C. japonicus, a number of potential conservation options will be identified, including the identification of ESUs: if they in fact exist. Examination of genetic structure will also substantially add to the current information available on this sole native Japanese species of freshwater crayfish, by providing effective populations sizes, sex ratios, and possibly measures of inbreeding or migration.

These genetic methods/techniques are non–lethal, reasonably inexpensive (US$15 per sample for standard DNA sequencing), very quick, tissue samples are easily transported via conventional mail, and samples can be stored for long periods of time without degrading. These analyses are routinely carried out in specialist laboratories in many regions of the world, and therefore remote collection sites, lack of equipment, and/or specialist expertise are not impediments to genetic techniques for the purposes of conservation.

In any case, the use of genetics in conservation efforts on freshwater crayfish can only aid in the prioritisation of allocating already scarce resources to conservations efforts and guide decision making processes towards the best possible outcomes.

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