

Implications of hybridization between introduced and resident

Orconectes crayfishes

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Running head: Hybridization between Crayfish

Word Count: 5683

Monday, August 13, 2001

Abstract

One of the most imperiled taxonomic groups in North American freshwaters is crayfish (Decapoda: Astacoidea) in which >30% of the 390 species are threatened or endangered. This situation is globally significant because 80% of the world's crayfish species are North American. Few studies have examined the environmental changes that most threaten crayfish biodiversity, but competition and hybridization with non-native crayfishes appear to be one of the most important threats to native crayfishes. The rusty crayfish, *Orconectes rusticus*, native to southeastern Indiana, has been introduced widely throughout the United States and is displacing two resident taxa, *O. propinquus* and *O. virilis*, in northern Wisconsin. Using morphological and allozyme comparisons of crayfish from allopatric and sympatric populations, we tested whether *O. rusticus* is hybridizing with the resident crayfishes in northern Wisconsin. We found no evidence for hybridization between *O. virilis* and either *O. rusticus* or *O. propinquus*. In contrast, numerous morphologically intermediate crayfish between *O. rusticus* and *O. propinquus* occurred at sympatric sites, and many of these individuals possessed allozymes diagnostic for both species in allopatry. Over 6% of the crayfish at one sympatric were putative F₁ hybrids, 4% were putative F₂ individuals (hybrid x hybrid origin), and 13% were putative backcrosses (product of hybrid x parental matings). This is the first genetic documentation of hybridization between a resident and invading crayfish. Our results suggest that genetic mechanisms play a role in the extirpation of *O. propinquus* by *O. rusticus*, and are consistent with observations of other researchers suggesting that hybridization with non-native species is common among crayfishes at many other locations. High rates of endemism and widespread introductions of non-native crayfish suggest that invasions and hybridization are a major threat to crayfish biodiversity.

Introduction

Recent work suggests that hybridization and introgression are major mechanisms by which non-indigenous species change or eliminate endemic gene pools (Rhymer & Simberloff 1996). The loss of endemic gene pools is most likely for smaller populations that come into contact with larger, introduced populations. Thus, species that are already reduced in number are most likely to suffer from hybridization and introgression, as several recent examples illustrate. Hybridization and introgression have contributed to declines in many imperiled species from a diversity of taxonomic groups, as the following examples illustrate: many species of native North American sunflower (*Helianthus*) (from hybridization with *H. annuus*); Catalina Island mahogany (*Cercocarpus traskiae*) (from hybridization with *C. betuloides*); New Zealand grey duck (*Anas superciliosa superciliosa*), Hawaiian duck (*A. wyvilliana*), Florida mottled duck (*A. fulvigula fulvigula*), Pacific black duck (*A. superciliosa rogersi*) (all from hybridization with the widely introduced mallard duck, *A. platyrhynchos*); the European wildcat (*Felis silvestris*) (from hybridization with feral housecats, *F. catus*); the North American red wolf (*Canis rufus*) [from hybridization with gray wolf (*C. lupus*) and coyote (*C. latrans*)]; Scottish red deer (*Cervus elaphus*) (from hybridization with Japanese sika deer, *C. nippon nippon*); Spanish frog (*Rana perezi*) (from hybridization with *R. ridibunda*); and Apache trout (*Oncorhynchus apache*) (from hybridization with rainbow trout, *O. mykiss*) (Rhymer and Simberloff 1996). Furthermore, the conservation significance of hybridization and introgression is certainly underestimated because many ecological studies rely only on morphological criteria, and it can be difficult to identify hybrid phenotypes. However, recent advances in molecular techniques provide powerful tools to identify individuals

genetically, and allow a re-evaluation of the importance of genetic mechanisms during species invasions and subsequent loss of native biodiversity (Rhymer & Simberloff 1996).

Hybridization and introgression with non-native species appear to be particularly prevalent in freshwater ecosystems, as indicated by many studies on fishes (Miller et al. 1989, Allendorf and Leary 1988). The North American freshwater fauna already suffers extinction rates estimated to be the highest of any global ecosystem (Ricciardi & Rasmussen 1999), and non-indigenous species are a major (often primary) cause of declining freshwater biodiversity (Lodge in press; Poff et al. in press; Sala et al. 2000). Long-recognized mechanisms by which non-indigenous species reduce native flora and fauna include habitat change, competition, predation, and parasitism (Lodge 1993a, b; Williamson & Fitter 1996), but hybridization and introgression are increasingly recognized as an important interacting factor with these better documented mechanisms.

Crayfish may be particularly prone to hybridization due to many recent introductions of non-indigenous taxa (Taylor et al. 1996; Lodge et al. 2000a) and low levels of genetic divergence among species (Brown 1981; Attard & Pasteur 1984; Agerberg 1990). Crayfishes are of major North American conservation concern for the following reasons: North America contains 75% of the world's crayfishes; >30% of the 390 North American crayfishes are considered threatened or endangered; and crayfishes are often keystone species in many freshwater food webs (Lodge et al. 2000a). Thus, any potential threat to native crayfish biodiversity is also a threat to freshwater community structure and ecosystem function.

The effects of non-indigenous species, including hybridization, are often implicated as causative factors in crayfish declines (Lodge et al. 2000a). Previous morphological analyses have implicated hybridization between certain crayfish (Crocker & Barr 1968; Capelli & Capelli 1980; Smith 1981; Berrill 1985; Page 1985), but genetic studies have not been conducted to verify the phenomenon in nature. One particularly well-studied invasion involves the rusty crayfish (*Orconectes rusticus*), which has become a serious pest in parts of eastern North America in the last 30 years.

Orconectes rusticus is endemic to the tributaries of the Ohio River in southwestern Ohio, northern Kentucky, and southeastern Indiana (U.S.A.). Due to its use as fishing bait, *O. rusticus* has spread as far north as Maine, (U.S.A.) and Ontario (Canada), south to Tennessee and west to New Mexico, (U.S.A.) (Hobbs & Jass 1988). Wherever the rusty crayfish has become established, it has extirpated native crayfish and disrupted local aquatic ecosystems (Butler & Stein 1985; Butler 1988; Lodge et al. 1994, 1998, 2000a). This has led to restrictions on the use of *O. rusticus* as fish bait in several states and calls for bans on the use of all live crayfish as bait (Lodge et al. 2000b).

Orconectes rusticus populations in northern Wisconsin and Michigan lakes have been studied extensively (Lodge et al. 1994, 1998). *Orconectes rusticus* was introduced to the region in the 1960s and is in the process of displacing two resident taxa, *O. propinquus* and *O. virilis* (Olsen et al. 1991). Ecological and demographic factors have contributed to the success of the rusty crayfish in northern Wisconsin and Michigan (Hill & Lodge 1999). *Orconectes rusticus* grows faster and reaches a larger body size than *O. propinquus* (Hill et al. 1993). Although *O. rusticus* and *O. virilis* are similar in size, *O. rusticus* has relatively larger chelae than *O. virilis* (Lodge et al. 1986). These features make *O. rusticus* less susceptible to predation (DiDonato & Lodge 1993; Garvey et al. 1994) and better able to secure food and shelter in competition with *O. propinquus* and *O.*

virilis (Capelli & Munjal 1982; Hill & Lodge 1998). *Orconectes rusticus* is also more fecund than *O. propinquus* (Corey 1987).

However, ecology and demography may not be the only factors driving the extirpation of local species by *O. rusticus*. Morphological studies suggest that *O. rusticus* is hybridizing with resident crayfish species (Crocker & Barr 1968; Capelli & Capelli 1980; Smith 1981). Yet, significant morphological variation among *O. rusticus* specimens from different localities made it difficult to definitively establish that these intermediate individuals were hybrids, rather than intraspecific variants stemming from environmental heterogeneity among lakes (Capelli & Capelli 1980; Tierney 1982).

We tested for hybridization between *O. rusticus*, *O. propinquus*, and *O. virilis* through an allozyme survey of crayfish from mono- versus multispecies assemblages in the midwestern United States. We report evidence for extensive interbreeding between *O. rusticus* and *O. propinquus*, but not between *O. virilis* and the other two taxa. Our data also suggest that *O. rusticus* x *O. propinquus* hybrids are fertile and largely backcross with *O. rusticus*. Therefore, genetic mechanisms probably contribute to the displacement of *O. propinquus* by *O. rusticus*. Our study is the first to document hybridization associated with the invasion and displacement of a resident crayfish species. Given the high biodiversity and geographic proximity of many crayfish species in the eastern United States and the frequent use of live crayfish as fish bait, closely related crayfish taxa may be at a high risk of extirpation through hybridization and other interactions with non-native crayfishes.

Methods

Overview of Study Design

We compared the morphology and genetics of crayfish from allopatric versus sympatric populations of *O. rusticus*, *O. propinquus*, and *O. virilis*. We define *allopatric* as monospecific demes of crayfish spatially disjunct from other populations. *Sympatric* refers to populations sampled from a lake or stream that contained two or more species. Our rationale was that if *O. rusticus* is actively hybridizing with either of the two resident crayfish, then sympatric populations should contain individuals with intermediate morphologies. Furthermore, these intermediate individuals should possess diagnostic genetic markers from both hybridizing taxa.

Sampling Scheme

We collected crayfish from 30 different sites in the midwestern United States (Table 1, Fig. 1). Sixteen of the 30 collecting sites were lakes and streams with no known history of more than one crayfish species and were considered to have allopatric populations, and 14 sites contained multispecies assemblages and were considered sympatric. For *O. rusticus*, only the 7 sites sampled from tributaries of the Ohio River in the states of Ohio, Indiana, and northern Kentucky were considered allopatric (Table 1, Fig. 1). This region is outside the known distribution of both *O. virilis* and *O. propinquus* (Fig. 1), and we assumed these collections were composed of pure *O. rusticus*. Although we also sampled monospecific populations of *O. rusticus* in northern Wisconsin and Michigan (Table 1, sites 29-30), these populations were introduced and probably had prior contact with *O. propinquus* and/or *O. virilis* (Olsen et al. 1991). Both sympatric and allopatric populations of *O. propinquus* and *O. virilis* were collected in northern Wisconsin and Michigan, depending upon whether or not they co-occurred with *O. rusticus*. Although there is not a consensus on the original range of *O. rusticus* (Creaser 1931, Page 1985,

Taylor in press) or the current distribution of *O. propinquus* (Page 1985, Hobbs & Jass 1988), the geographic areas of disagreement do not include the areas of our study.

Crayfish were collected by hand from all sites except Trout Lake, where we used standard trapping techniques as outlined in Olsen et al. (1991). Abdominal (tail) tissue was dissected from each crayfish and stored in liquid nitrogen for later genetic analysis. Carcasses were also frozen prior to morphological analysis of the exoskeleton. We used only sexually mature females and males (>1 year old). Results from the morphological analysis are presented for males only because all taxonomic keys are based on males and there are no reliable quantitative morphological features that unambiguously separate females of these three species although qualitative features can be used. *Orconectes* in the midwestern United States generally reach sexual maturity after 1 year, with males being in a sexually competent form (form I) from late summer to early spring and in a sexually incompetent form (form II) during the remainder of the year. In this study we analyzed both form I and form II males morphologically and genetically. Because the morphological indices we evaluated in this study did not differ between form I and form II males (statistical analyses not shown), we report the pooled results for both form I and form II males. Both sexes were included in the genetic survey testing for hybridization.

Morphological Measurements

Morphological measurements were taken for 12 different characters from the exoskeletons of male crayfish (Fig. 2). The traits were measured to the nearest ± 0.01 mm using a Mitutoyo (model CD-S6" C) digital caliper and a dissecting microscope (12X) with an ocular micrometer. We did not measure morphological features that were regenerating from previous damage. We used discriminant function analysis (DFA; Manly 1994; Wilkinson 1997) to examine the extent of morphological overlap between *O. rusticus*, *O. propinquus*, and *O. virilis* specimens collected from allopatric populations. All 12 morphological features (Fig. 2) were included in the DFA to define linear functions that best separated individuals into the three predetermined species groups. A jackknife procedure was then performed to assess the accuracy of the DFA linear equations. Jackknifing involved randomly eliminating one individual from the data set and then recalculating the DFA based on the revised information. The resulting linear equations were subsequently used to reclassify the eliminated individual. This procedure was repeated until every specimen had been deleted once from the data set. As an estimate of the accuracy of the DFA, we report the percentage of instances in which the removed individual was correctly reclassified. The DFA was used in the same manner for sympatric populations to determine if taxa were morphologically less distinct in sympatry than allopatry.

Genetic Analysis

Crayfish were scored for the following enzyme systems using standard horizontal starch gel electrophoretic techniques (Murphy et al. 1990): aconitase (*Acon*), adenylate kinase (*Ak*), aspartate aminotransferase (*Aat*), fructose-bisphosphatase (*Fbp*), glucosephosphate isomerase (*Gpi*), glyceraldehyde-3-phosphate dehydrogenase (*G-3-pdh*), hydroxyacid dehydrogenase (*Had*), isocitrate dehydrogenase (*Idh*) and malate dehydrogenase (*Mdh*). *Acon*, *Ak*, *Aat*, *Fbp*, *G-3-pdh*, *Had*, and *Idh* were resolved using a 8 mM TRIS, 3mM citric acid monohydrate, pH 6.7 gel buffer, with a TRIS-citric acid electrode buffer (0.22M TRIS, 86 mM citric acid monohydrate, pH 6.3). The *Gpi* and *Mdh* were run using a 76 mM TRIS, 5 mM citric acid monohydrate, pH 8.7 gel buffer and a 0.3 M borate electrode buffer (pH 8.2). Isozyme systems were numbered in increasing

order according to the distance that they migrated from the cathode. The most common allele for each locus in *O. rusticus* was assigned a value of 100, and all other alleles were designated according to their anodal mobilities relative to this 100 allele. The maximum likelihood technique of Nason and Ellstrand (1993) was used to estimate the frequency of hybrid and backcross crayfish based on multilocus allozyme genotypes. This method assumes: 1) markers are codominant and their frequencies in the parental species are constant over time, 2) a population consists only of parental species and first- and second generation hybrid individuals, 3) mating is random within, and when it occurs, between genealogical classes, and 4) mendelian laws of inheritance apply - marker alleles are selectively neutral, and independently segregate and assort (Nason & Ellstrand 1993). If the model assumptions are violated, then estimates of the frequency of introgression tend to be conservative (Nason & Ellstrand 1993).

Results

Morphological Analysis of Allopatric Populations

Allopatric populations of *O. rusticus*, *O. propinquus* and *O. virilis* were morphologically distinct from one another (Fig. 3). The DFA with the jackknife reclassification procedure distinguished allopatric samples of males of the three species with 100% accuracy. Because not all traits were equally informative in differentiating the taxa, we present the two most informative traits: 1) distance between the central and mesial projection divided by the total gonopod length and 2) areola width divided by carapace length with which the DFA also distinguished allopatric samples of males of the three species with 100% accuracy (Fig. 3). The DFA unambiguously classified males from allopatric populations based on these two characters alone (Fig 3).

Genetic Analysis of Allopatric Populations

Two of the nine allozyme systems, *Ak* and *Acon*, were monomorphic and fixed for the same allele in all populations of *O. rusticus*, *O. propinquus*, and *O. virilis*, making them taxonomically uninformative. Two other allozymes, *Gpi* and *Mdh*, were polymorphic within at least one of the three species. These systems, however, were not diagnostic because allopatric populations of *O. rusticus*, *O. propinquus* and *O. virilis*, still shared a majority of alleles for these loci. Five systems, *Aat*, *Fbp*, *G-3-pdh*, *Had*, and *Idh*, were species specific, with different alleles fixed in allopatric populations of at least one species (Table 2). Unfortunately, *Fbp* could not always be reliably scored from abdominal tail tissue due to technical difficulties. The allozymes *Had*, *Idh*, *Aat*, and *G-3-pdh* therefore formed the basis for our genetic test of hybridization between sympatric populations of *O. rusticus*, *O. propinquus* and *O. virilis*.

Morphological and Genetic Analysis of Sympatric Populations

Analysis of sympatric sites revealed no evidence for hybridization or introgression between *O. virilis* and either *O. rusticus* or *O. propinquus*. As was the case in allopatry, *O. virilis* was morphologically and genetically distinct from *O. rusticus* and *O. propinquus* in sympatric assemblages (Fig. 4). The alleles *G-3-pdh* 90 and *Aat* 90 were diagnostically fixed in both allopatric and sympatric populations of *O. virilis*. No crayfish from a sympatric site possessed a unique allele for *O. virilis* together with an allele unique to either *O. rusticus* or *O. propinquus*. In addition, *O. virilis* from sympatric sites were morphologically similar to *O. virilis* from allopatric populations (Fig. 4). The apparently greater morphological variation in sympatric *O. virilis* populations relative to allopatric populations (several gray squares in Fig. 4 fall outside the polygons derived

from the allopatric sites in Fig. 3) can be explained by the increased sample size from sympatric relative to allopatric populations (Table 1).

In contrast to the results for *O. virilis*, there was unequivocal evidence for hybridization and introgression between *O. rusticus* and *O. propinquus*. Many crayfish at sympatric sites had morphologies intermediate between those observed for *O. rusticus* and *O. propinquus* in allopatry (Fig. 4). As a consequence, DFA was much less effective in distinguishing morphologically identified *O. rusticus* from *O. propinquus* at sympatric (80% accuracy) than allopatric sites (100% accuracy). The increase in morphological diversity observed in sympatry could not be explained solely on the basis of expanded sampling because many of the intermediate individuals possessed diagnostic allozymes from each species, indicative of hybrid ancestry (Fig. 4A). Regression analysis revealed a highly significant relationship between a genetic hybrid index score based on the number of diagnostic *O. rusticus* *Idh* 100 and *Had* 100 alleles that crayfish possessed and the morphological character of central - mesial projection divided by total gonopod length ($R^2 = 0.782$, $p < 0.0001$). About 7.5 % of the total sample from sympatric sites were heterozygous for both *Idh* and *Had*, making them “putative” F₁ hybrids (Fig. 4A). These individuals are referred to as putative F₁s because with only two diagnostic loci, it is likely that a portion of them are actually F₂s, backcrosses, or later generation backcrosses. About half of the putative F₁ hybrids had phenotypes outside of the ranges of *O. rusticus* and *O. propinquus*, although the overall F₁ morphological distribution was skewed toward *O. rusticus* (Fig. 4A). Crayfish that had three of a possible four alleles diagnostic for *O. rusticus* at *Idh* and *Had* were morphologically similar to *O. rusticus* (Fig. 4D), as were individuals homozygous for *O. rusticus* alleles at one locus and *O. propinquus* alleles at the other (Fig. 4C). In contrast, individuals possessing one *O. rusticus* allele and three *O. propinquus* alleles spanned almost the entire morphological range for the two species (Fig. 4B). These findings suggest *O. rusticus* genes may, in general, be dominant with respect to morphology. Additional nuclear markers, however, must be resolved to rule out genetic misclassification as a factor (i.e., our characterization of the proportion of an individual’s genome comprised of *O. rusticus* vs. *O. propinquus* genes based on two allozymes may not always be accurate).

Discussion

Our results for *O. propinquus* and *O. rusticus* represent the first genetically verified example of hybridization in crayfish. The strong correspondence between morphology and allozyme markers at sympatric sites confirms the suspicion of Capelli and Capelli (1980) that these two taxa interbreed in nature. Genetic mechanisms therefore contribute to the extirpation of *O. propinquus*, although the relative importance of genetic versus ecological and demographic factors remains to be determined. In contrast, populations of *O. virilis* are as morphologically and genetically distinct from *O. rusticus* and *O. propinquus* in sympatry as they are in allopatry. Ecological and demographic mechanisms are therefore sufficient to explain the local extinction of *O. virilis* by *O. rusticus* (Hill & Lodge 1999).

Hybridization appears fairly common, and introgression appears to be extensive between *O. rusticus* and *O. propinquus*. Because only two diagnostic allozymes currently distinguish the taxa, our ability to ascertain the genetic ancestry of individual crayfish is somewhat compromised. Nevertheless, maximum likelihood techniques can still be applied to our data to estimate hybridization and introgression frequencies (Nason & Ellstrand 1993). Our maximum likelihood estimate for the percentage of F₁ hybrids in

Trout Lake, the site with the largest sample size ($n = 781$), was 6.3 ± 2.7 % (95% confidence interval determined by bootstrapping over 10,000 replicates). The F_2 and backcross crayfish were estimated to comprise 3.7 ± 0.4 % and $13.4\% \pm 7.2$ % of the population, respectively.

Despite the non-trivial proportions of crayfish of mixed ancestry in Trout Lake, it would probably be inaccurate to describe this population as a hybrid swarm. First, detailed trapping data indicate that *O. rusticus* is advancing at a rate of $\sim 0.7 \pm 0.1$ km per year along the shoreline of Trout Lake (Perry et al. unpublished data). Second, the production of intermediate phenotypes ceases and the frequency of crayfish of mixed ancestry decreases after *O. propinquus* are extirpated from a region of the Lake (Perry et al. unpublished data). Consequently, the hybrid zone between *O. rusticus* and *O. propinquus* does not appear to be spatially or temporally stable.

Capelli and Capelli (1980) and Smith (1981) suggest that hybrids between *O. rusticus* and *O. propinquus* were not viable because of the absence of a hybrid swarm and backcross individuals. However, the existence of numerous crayfish of F_2 and backcross ancestry (combined total ≈ 17 % of the Trout Lake population based on maximum likelihood estimate) implies hybrids are fertile. Unfortunately, we are unable to draw any quantitative conclusions about the relative viabilities and fertilities of hybrids, F_2 s and backcrosses from our data. Nevertheless, our estimate that 6.3 % of crayfish at Trout Lake are F_1 hybrids, using the maximum likelihood technique, suggests that hybrids do not suffer inordinate mortality compared to parental types. We are currently comparing fecundity and survival of different genotypes in the field and laboratory to address this issue. Even if hybrids and backcrosses have reduced fecundities relative to parental species, the present data suggest their fertility is high enough to result in detectable levels of introgression between *O. rusticus* and *O. propinquus*.

The maximum likelihood solution for F_2 backcrosses in Trout Lake suggests that introgression is directional and primarily involves the movement of *O. propinquus* alleles into *O. rusticus*. We estimate that 0.9 ± 2.7 % (95% confidence interval) of the total crayfish population are the products of F_1 hybrids backcrossing to *O. propinquus*, whereas 12.5 ± 4.5 % represent backcrosses to *O. rusticus*. The F_1 hybrids therefore appear to mate disproportionately with pure *O. rusticus*, as the ratio of pure parental types in the lake was estimated as only 61% *O. rusticus* vs. 19% *O. propinquus*. However, additional data are needed to rule out viability and fecundity differences among genotypes as contributing factors to the pattern. Regardless of the cause for the pattern, the practical consequences are that genetic introgression is much greater from *O. propinquus* into *O. rusticus* than in the reverse direction.

The long-term evolutionary consequences of introgression are unclear because *O. propinquus* alleles may still be selected against (disfavored) in an *O. rusticus* genetic background. If such selection is occurring, then eventually *O. propinquus* genes may gradually be eliminated from the population. This is another aspect of the system that requires further study. We can say that certain populations such as Bond Falls, Ontonagon River and Roselawn Creek (Table 1, sites 28 - 30) currently have few or no *O. propinquus* alleles. In contrast, other sites where *O. rusticus* has invaded and displaced previously known *O. propinquus* populations contain ample genetic remnants of *O. propinquus*. For example, Birch Lake (Table 1, site 27) is inhabited exclusively by what morphologically appears to be pure *O. rusticus*. Nevertheless, *O. propinquus* alleles were found in 25% of the individuals in the lake. Thus, it is possible for *O. propinquus* genes

to persist for at least some time following invasion by *O. rusticus*. Resolution of the introgression issue will clarify whether the *O. rusticus* interaction with *O. propinquus* is best described as genetic assimilation, as opposed to genetic extirpation.

Given the extensive hybridization occurring between *O. rusticus* and *O. propinquus*, our study raises questions about what constitutes a crayfish species. *Orconectes rusticus* and *O. propinquus* are morphologically and genetically distinct entities in allopatry (Fig. 3, Table 2). However, in zones of sympatry in northern Wisconsin lakes these differences are not maintained (Fig. 4). Wherever the two taxa meet, *O. rusticus* ecologically displaces and, through hybridization, genetically assimilates and morphologically extirpates *O. propinquus* populations. Thus, under the biological species concept, *O. rusticus* and *O. propinquus* do not represent "good" species; they are not reproductively isolated entities, but rather combine into a single population (taxon) in sympatry. Perhaps it is most accurate to view *O. rusticus* and *O. propinquus* as geographic subspecies. As we discuss below, our results most likely apply to other crayfishes as well. Some, perhaps many, of the 390 crayfish taxa currently recognized as species in the North America may not be recognized as such under the biological species concept.

However, from a conservation standpoint the biological species concept may not be the most relevant criterion for determining species status (Arnold 1997). Although *O. rusticus* and *O. propinquus* have not diverged to the point that they can permanently co-exist in sympatry, they are not ecologically equivalent. Wherever *O. rusticus* has displaced *O. propinquus*, *O. rusticus* has changed freshwater ecosystems. For example, *O. rusticus* significantly reduces both the biomass and diversity of macrophytes and algae in lakes and streams (Lodge & Lorman 1987; Lodge et al. 1994; Charlebois & Lamberti 1996, Lodge et al. 2000). *Orconectes rusticus* also reduces the density and diversity of snails and other macroinvertebrates through predation (Olsen et al. 1991; Charlebois & Lamberti 1996; Lodge et al. 1998; Perry et al. 1997; Lodge et al. 2000). Finally, crayfish comprise a significant proportion of the diets of many fish (Rabeni 1992; Roell & Orth 1992), but *O. rusticus* is less preferred than *O. propinquus* (DiDonato & Lodge 1993; Garvey et al. 1994). Thus, through littoral habitat changes and reductions in invertebrate prey and fish nest disruptions, the introduction of *O. rusticus* into northern Wisconsin and Michigan is thought to be a significant factor contributing to some declining sport fisheries in the region (Lodge et al. 1985). From a conservation perspective, it therefore matters more that *O. rusticus* and *O. propinquus* are not ecologically equivalent and matters less that they do not conform to the biological species concept. This suggests that phylogenetic species definitions may often be more useful to conservation biologists than the biological species concept, with the caveat that the unique morphological and genetic autapomorphies that these taxa possess translate into ecological differences. Consequently, the extirpation of *O. propinquus* causes not only the local loss of native biodiversity, but large changes in freshwater ecosystems.

We have documented one instance where hybridization is playing a role in a crayfish invasion, but we believe this process is common for crayfishes. Morphological evidence from several researchers suggests that hybridization regularly occurs within and between crayfish subgenera: *O. virilis* (*Gremicambarus*) and *O. immunis* (*Gremicambarus*) (D. Jensen personal communication); *O. obscurus* (*Crockerinus*) and *O. propinquus* (*Crockerinus*) (Capelli & Capelli 1980); *O. obscurus* and *O. rusticus* (*Procericambarus*) (Capelli & Capelli 1980); *O. rusticus* and *O. jeffersoni* (*Crockerinus*)

(C. Taylor personal communication); and *O. rusticus* and *O. sanbornii* (Crockerinus) (Butler & Stein 1985). Genetic analysis is needed in the aforementioned cases to confirm hybridization. Nevertheless, the implication is that hybridization and introgression pose a substantial threat to the conservation of crayfish and freshwater ecosystems. Thus, our study is consistent with the thesis of Rhymer and Simberloff (1996) that hybridization and introgression are much more common components of threats to biodiversity and ecosystem function than is recognized.

Acknowledgments

We thank M. Schmitz and J. Roethele for help in collecting and analyzing the samples and the University of Wisconsin, Madison, Wisconsin's Trout Lake LTER for providing lab space. This research was supported by a cooperative agreement with the U.S. Environmental Protection Agency (CR820290-02-0) and by a graduate research traineeship to WLP from the National Science foundation under grant no. 9452655. This research was a contribution of the University of Notre Dame Environmental Research Center.

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Table 1. Collecting site information for *O. rusticus*, *O. propinquus*, and *O. virilis*.

No.	Location ^a	A/S ^b	Lat. (N)	Long. (W)	Males ^c					Both Sexes ^c						
					n	OR	OP	hyb	back	OV	n	OR	OP	hyb	back	OV
1	Bank Lick Cr., KY	A	39°02'	84°29'	19	100	-	-	-	-	35	100	-	-	-	-
2	Big Blue R., IN	A	39°20'	85°59'	10	100	-	-	-	-	17	100	-	-	-	-
3	Cherry Fork Cr., OH	A	38°55'	83°39'	20	100	-	-	-	-	33	100	-	-	-	-
4	Dry Fork Cr., OH	A	39°15'	84°45'	4	100	-	-	-	-	7	100	-	-	-	-
5	Flatrock Cr., IN	A	41°10'	84°26'	2	100	-	-	-	-	2	100	-	-	-	-
6	Garrison Cr., KY	A	39°06'	84°48'	5	100	-	-	-	-	8	100	-	-	-	-
7	Shaker Cr., OH	A	39°28'	84°21'	2	100	-	-	-	-	2	100	-	-	-	-
8	Allequash L., WI	A	46°02'	89°37'	7	-	100	-	-	-	10	-	100	-	-	-
9	Manitowish R., WI	A	46°07'	89°39'	7	-	100	-	-	-	14	-	100	-	-	-
10	Mill Cr., MI	A	42°11'	86°15'	3	-	100	-	-	-	6	-	100	-	-	-
11	Great Br., NY	A	42°58'	77°23'	20	-	100	-	-	-	35	-	100	-	-	-
12	Tenderfoot Cr., MI	A	46°13'	89°31'	4	-	100	-	-	-	9	-	100	-	-	-
13	Tamarak R., MI	A	46°03'	89°16'	2	-	-	-	-	100	4	-	-	-	-	100
14	Whitefish L., MI	A	45°54'	89°51'	22	-	-	-	-	100	36	-	-	-	-	100

Table 1. Cont.

No.	Location ^a	A/S ^b	Lat. (N)	Long. (W)	Males ^c					Both Sexes ^c						
					n	OR	OP	hyb	back	OV	n	OR	OP	hyb	back	OV
15	Pine R., WI	A	46°34'	89°13'	3	-	-	-	-	100	18	-	-	-	-	100
16	M. Br. Wisc. R., WI	A	44°54'	89°29'	10	-	-	-	-	100	35	-	-	-	-	100
17	Ontonagon R., MI	S	46°27'	89°19'	26	96	4	-	-	-	38	97	3	-	-	-
18	Plum Cr., WI	S	45°56'	89°32'	51	29	71	-	-	-	76	37	60	3	-	-
19	Long L., WI	S	46°04'	89°00'	7	-	100	-	-	-	11	-	98	-	-	2
20	Van Vliet L., WI	S	46°11'	89°45'	12	92	-	-	-	8	12	92	-	-	-	8
21	White Sand L., WI	S	46°05'	89°35'	34	33	-	6	29	32	35	34	-	6	29	31
22	Allequash Cr., WI	S	46°02'	89°36'	45	69	27	2	2	-	71	70	20	3	7	-
23	Little John L., WI	S	46°00'	89°38'	10	-	30	-	40	30	10	-	30	-	40	30
24	Plum L., WI	S	46°00'	89°30'	29	62	17	14	-	7	29	62	17	14	-	7
25	S. Turtle L., WI	S	46°12'	89°53'	53	34	17	21	26	2	53	34	17	21	26	2
26	Trout L., WI	S	46°02'	89°40'	546	58	18	10	10	4	781	61	19	9	8	3
27	Birch L., WI	S	46°13'	89°50'	12	75	-	8	17	-	12	75	-	8	17	-
28	Bond Falls, MI	S	46°23'	89°06'	18	94	-	-	6	-	18	94	-	-	6	-

Table 1. Cont.

No.	Location ^a	A/S ^b	Lat. (N)	Long. (W)	Males ^c					Both Sexes ^c						
					n	OR	OP	hyb	back	OV	n	OR	OP	hyb	back	OV
29 ^d	Ontonagon. R., MI	S	46°14'	89°09'	12	100	-	-	-	-	27	100	-	-	-	-
30 ^d	Roselawn Cr., MI	S	46°24'	89°12'	19	100	-	-	-	-	39	100	-	-	-	-

^a State Abbreviations are: IN – Indiana, KY - Kentucky, MI – Michigan, OH – Ohio, and WI – Wisconsin

^b Sites were classified as A, allopatric, or S, sympatric.

^c The percentages of pure *O. rusticus* (OR), *O. propinquus* (OP) and *O. virilis* (OV) crayfish at sites, and putative *O. rusticus* x *O.*

propinquus F1 hybrids (hyb) and backcrosses (back) are based on the genetic survey of allozyme loci. Genetic categories and sample

sizes (n) are given for males and both sexes pooled and reflect raw genotype numbers and not maximum likelihood estimates.

^d Sites 29 and 30 are categorized as sympatric because these populations were introduced and likely had prior contact with *O.*

propinquus and/or *O. virilis* which are found inflowing and outflowing streams.

Table 2. Diagnostic allozymes resolved for allopatric populations of *O. rusticus* (OR), *O. propinquus* (OP), and *O. virilis* (OV).

Enzyme(EC number)	Abbreviation	Diagnostic allozyme allele		
		OR	OP	OV
Isocitrate dehydrogenase (1.1.1.42)	<i>Idh</i>	100	90	90
D-2-Hydroxy-acid dehydrogenase (1.1.99.6)	<i>Had</i>	100	110	100
Aspartate aminotransferase (2.6.1.1)	<i>Aat</i>	100	100	90
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	<i>G-3-pdh</i>	100	100	90
Fructose-bisphosphatase (3.1.3.11)	<i>Fbp</i>	100	120	110

Figure Legends

Figure 1. The current geographic distributions, from the most recently published sources, for *O. rusticus* (Page 1985, Taylor in press), *O. propinquus* (Hobbs & Jass 1988), and *O. virilis* (Hobbs & Jass 1988) in the midwestern United States. The probable original range for *O. rusticus* is also shown based on Page (1985). Identification numbers of collection locations for all species are shown on the *O. propinquus* subfigure and are keyed to site information in Table 1.

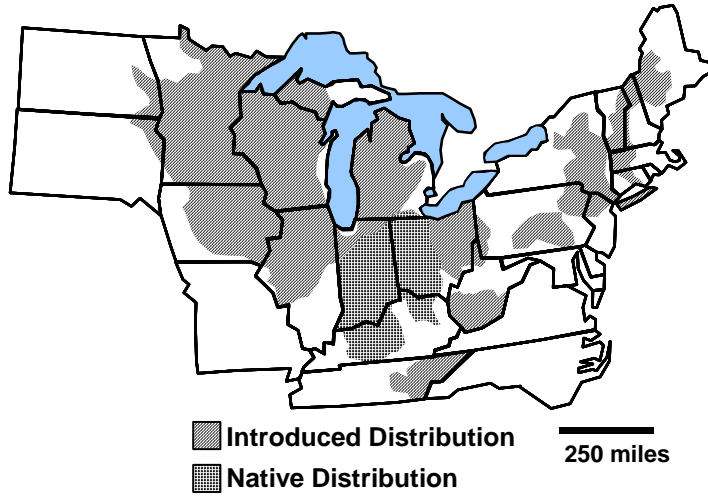
Figure 2. Morphological features measured (millimeters) for male crayfish; 1) carapace length, 2) areola length, 3) areola width, 4) carapace width, 5) acumen length, 6) rostral width, 7) chelae length, 8) dactyl length, 9) palm width, 10) gonopod length, 11) central projection length, and 12) mesial projection length.

Figure 3. Morphological traits differentiating allopatric populations of male *O. rusticus*, *O. propinquus*, and *O. virilis*. Areola width divided by carapace length is plotted along the y - axis against the difference in length between central and mesial projections divided by total gonopod length along the x-axis. Polygons enclose the morphological area occupied by each of the three taxa and were added only to allow convenient comparison with Fig. 4.

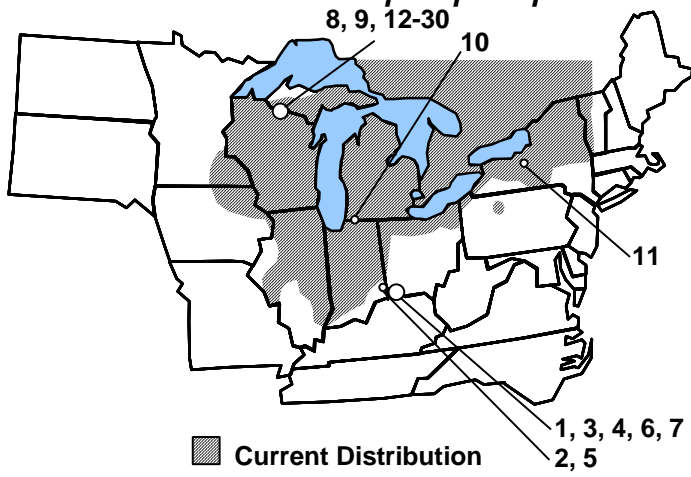
Figure 4. Morphological scores for male crayfish belonging to different genotypic categories at sympatric sites. Areola width divided by carapace length is plotted along the y - axis against the difference in length between central and mesial projections divided by total gonopod length along the x-axis. A) Comparison of individuals genetically typed as

pure *O. rusticus* (shaded circles), *O. propinquus* (shaded triangles) and *O. virilis* (shaded squares) with putative F₁ hybrids between *O. rusticus* and *O. propinquus* (black circles). Crayfish that were double heterozygotes for the allozymes *Had* and *Idh* were classified as F₁ hybrids, while individuals homozygous for species specific alleles at both loci were assigned to the appropriate parental class. B) Comparison of parental genotypes (as in Fig. A above) with putative F₂ and backcross possessing one *O. rusticus* allele and three *O. propinquus* alleles at *Had* and *Idh* (black circles). C) Comparison of parental genotypes (as in Fig. A above) with putative F₂ individuals homozygous for two *O. rusticus* alleles at one locus (*Had* or *Idh*) and two *O. propinquus* alleles at the other. D) Comparison of parental genotypes (as in Fig. A above) with putative F₂ and backcross possessing three *O. rusticus* alleles and one *O. propinquus* allele. Overlaid polygons represent the range of morphological variation found for the three taxa from allopatric populations (See Fig. 3).

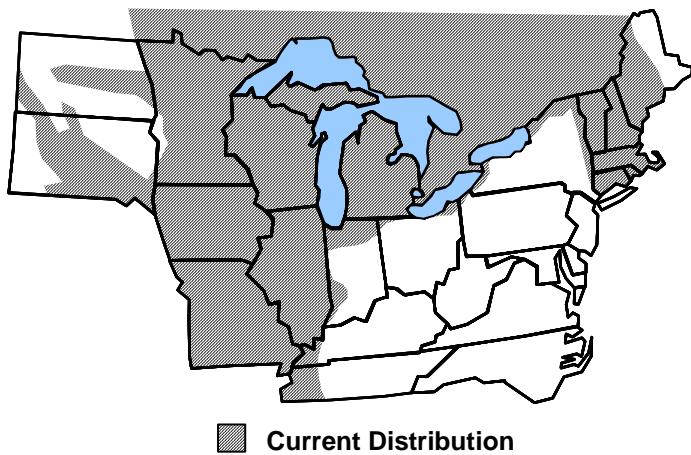
Orconectes rusticus



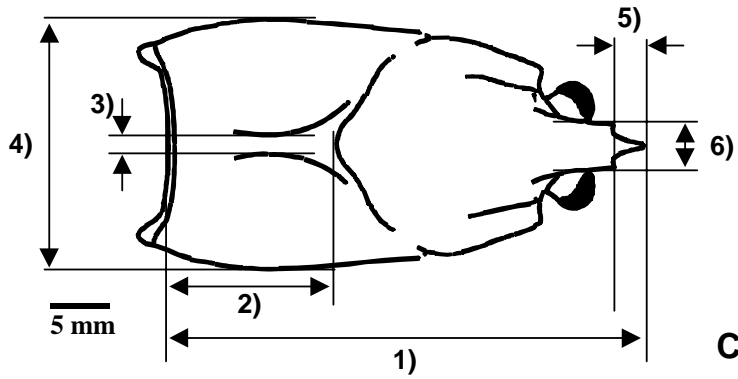
Orconectes propinquus



Orconectes virilis



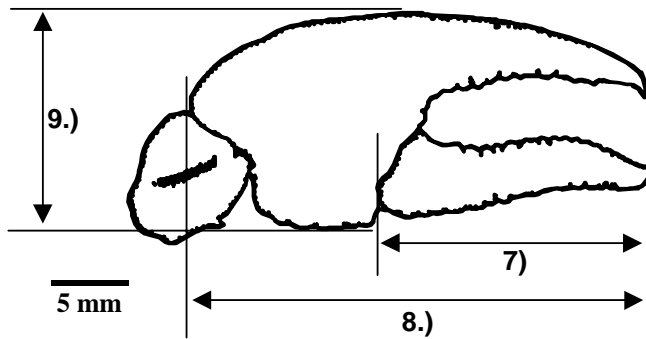
Cephalothorax



Cephalothorax

- 1) Carapace Length
- 2) Areola Length
- 3) Areola Width
- 4) Carapace Width
- 5) Acumen Length
- 6) Rostral Width

Chela



Chela

- 7) Dactyl Length
- 8) Chelae Length
- 9) Palm Width

Gonopod

- 10) Gonopod Length
- 11) Central Projection Length
- 12) Mesial Projection

Gonopod

