J. Parasitol., 93(3), 2007, pp. 575–582

GENETIC DIVERSITY AND EXPANDING NONINDIGENOUS RANGE OF THE RHIZOCEPHALAN *LOXOTHYLACUS PANOPAEI* PARASITIZING MUD CRABS IN THE WESTERN NORTH ATLANTIC

Inken Kruse* **and Matthew P. Hare**
Department of Biology, University of Maryland, College Park, Maryland 20742. *e-mail: inkenkruse@web.de*

ABSTRACT: Nonindigenous parasite introductions and range expansions have become a major concern because of their potential to restructure communities and impact fisheries. Molecular markers provide an important tool for reconstructing the pattern of introduction. The parasitic castrator *Loxothylacus panopaei*, a rhizocephalan barnacle, infects estuarine mud crabs in the Gulf of Mexico and southeastern Florida. A similar parasite introduced into Chesapeake Bay before 1964, presumably via infected crabs associated with oysters from the Gulf of Mexico, was identified as *L. panopaei*. Our samples of this species during 2004 and 2005 show that the introduced range has expanded as far south as Edgewater, Florida, just north of the northern endemic range limit. The nonindigenous range expanded southward at a rate of up to 165 km/yr with relatively high prevalence, ranging from 30 to 93%. Mitochondrial DNA sequences from the cytochrome oxidase I gene showed that these nonindigenous *L. panopaei* are genetically distinct from the endemic parasites in southeastern Florida and the eastern Gulf of Mexico. The genetic difference was also associated with distinct host spectra. These results are incompatible with an eastern Gulf source population, but suggest that unrecognized genetic and phenotypic population structure may occur among Gulf of Mexico populations of *Loxothylacus*.

The introduction of nonindigenous species has become an intensely studied subject because the species pose disturbance and extinction risks within native communities (Simberloff et al., 2005). The predictability and repercussions of these effects are not without controversy, however, so invasion biology has rallied basic, as well as applied, research in an attempt to understand the species properties that promote long-distance transport and successful establishment. These studies help define community attributes that confer resistance against establishment of invasive taxa (reviewed in Pimm, 1991). Not surprisingly, invasive taxa with well-known taxonomy and established historical distribution records have received the most research attention (Streftaris et al., 2005).

A major impediment to the comparative analysis of invasion patterns is the large proportion of taxa that are cryptogenic (of hidden origin). These are species with no definite evidence of their native or introduced status (Carlton, 1996) or, in other words, hypothesized introductions with few data bearing on origins. Cryptogenic invasions have a high research priority when they have severe consequences (Burreson et al., 2000; Hoppe, 2002; Gozlan et al., 2005) or when they can be particularly instructive about biotic and abiotic mechanisms controlling invasion (e.g., Bastrop et al., 1998; Jousson et al., 1998; Blank et al., 2004). It is in this arena that molecular markers provide a crucial tool for the study of invasions, both because genetic diversity is an attribute that can change during invasion and may affect survival (Tsutsui et al., 2000; Lee, 2002), and because genetic polymorphisms provide tags that help trace invasion history (Geller et al., 1997; Jousson et al., 1998; Davies et al., 1999). Ironically, applying these powerful tools may initially increase the proportion of invasive taxa in the cryptogenic group by identifying false assumptions and historical inaccuracies, but even these revelations help us know what we don't know. In the present study, we used mitochondrial cytochrome oxidase I sequences to test specifically for genetic homogeneity between invasive and native populations of *L. panopaei*, a rhizocephalan parasite of mud crabs.

In marine systems, ecologically important but understudied groups such as parasites and bloom-creating phytoplankton, when introduced, are largely cryptogenic (Carlton, 1996; Torchin et al., 2002). Parasites and pathogens are a potentially devastating fraction of invasive species because their cascading effects can be rapid and severe (McCallum et al., 2003; Gozlan et al., 2005). We know little about the extent to which general patterns of marine invasion also hold for parasites, or how host–parasite interactions influence the propensity of parasites to become invasive (Torchin et al., 2002). Moreover, both macro- and microparasites are likely to contain hidden species (e.g., Huspeni, 2000), complicating attempts to identify their introduced status and biogeographic origins.

Sacculinid parasites of crabs have long been recognized as invasive (Boschma, 1972). These are rhizocephalan barnacle species in which female larvae infect recently molted crabs and proliferate internally, ultimately producing a sack or externa protruding from the crab abdomen. Male larvae then enter the externa and fertilize eggs (Høeg and Lützen, 1995). Infections persist through multiple crab molts, preventing the host from reproducing in an example of "parasitic castration" (Kuris, 1974; Alvarez et al., 1995). In the western North Atlantic, mechanisms determining the dispersal and invasiveness of sacculinid parasites are of great concern because the commercially important greater blue crab, *Callinectes sapidus*, is 1 of several portunid crabs infected by the castrating sacculinid *Loxothylacus texanus*. Blue crab commercial fisheries in the Gulf of Mexico are worth more than US$30 million annually (Guillory et al., 1998) and suffer from periods of high parasite prevalence (Shields and Overstreet, 2003). In contrast, blue crabs along the United States Atlantic coast are not infected by this parasite and in this region the commercial fishery in 2004 was worth US$101 million (NOAA Web site). It is not known what mechanisms prevent expansion of *L. texanus* northern range distribution to include mid-Atlantic host populations, but physical dispersal barriers were hypothesized, based on experiments showing that *L. texanus* larvae will settle on, i.e., recognize, *C. sapidus* from the Delaware Bay as well as from the Gulf of Mexico (Boone et al., 2004). Because the larval dispersal stage

Received 9 March 2006; revised 13 November 2006; accepted 13 November 2006.
* Present address: Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949.

of this parasite cannot survive salinities lower than 20 ppt (Tindle et al., 2004), it is conceivable that southeastern U.S. rivers create barriers to northern expansion. Possible counterevidence includes 1 report of *L. texanus* infection of blue crabs in South Carolina (Eldridge and Waltz, 1977, as cited in Shields and Overstreet, 2003).

Worldwide, there are 27 species of *Loxothylacus*, with eastern Asia harboring two-thirds of this species richness (Boschma, 1955; Reinhard and Reischman, 1958). A congener of *Loxothylacus texanus* also has its native range in the Gulf of Mexico and southern Florida. *Loxothylacus panopaei* (Gissler, 1884) infects species of xanthid mud crabs within a native range that extends into Atlantic Florida as far as Cape Canaveral (Hines et al., 1997). *Loxothylacus panopaei* was accidentally introduced into Chesapeake Bay where it became well established as a parasite of *Eurypanopeus depressus* and *Rhithropanopeus harrisii*, 2 broadly distributed xanthid crabs that also occur throughout the Gulf of Mexico (Van Engel et al., 1966). Sacculinid parasites had not previously been reported infecting crabs north of Cape Canaveral, Florida, in the western North Atlantic, with 1 exception (*Sacculina hirsuta* Boschma in North Carolina; Pearse and Williams, 1951), so the appearance of *L. panopaei* was likely to be anthropogenic. Van Engel et al. (1966) hypothesized that the introduction resulted from infected crabs contained within recent live oyster shipments from the Gulf of Mexico.

The roughly coincident native range limits for these congeneric crab parasites, and the agreement of these limits with a long-recognized zoogeographic province boundary near Cape Canaveral (Briggs, 1974), makes the successful invasion of Chesapeake Bay by *L. panopaei* both intriguing and foreboding with respect to potential range expansion of *L. texanus*.

Like *L. panopaei*, suitable hosts are available for *L. texanus* if it ever gets transported north of Florida. *Loxothylacus panopaei* infects at least 6 xanthid crab species and 1 goneplacid species (*Tetraplax quadridentata*) in its native range. Two of these native-range hosts, *Eurypanopeus depressus* and *Rhithropanopeus harrisii* (Reinhard and Reischman, 1958), are also present in Chesapeake Bay. It was not surprising, therefore, that *L. panopaei* infections were restricted to *E. depressus* in the York River, Virginia, in 1964 (Van Engel et al., 1966). Later, 1 new xanthid host was found infected by *L. panopaei* in Chesapeake Bay, i.e., *Dispanopeus sayi* in 1983 (Hines et al., 1997).

Physical dispersal barriers, or climate, or both, are likely factors determining the natural northern range limit at Cape Canaveral of *L. texanus* and *L. panopaei* (Gaston, 2003). The transition between temperate and subtropical marine fauna in eastern Florida is coincident with a relatively steep temperature gradient (Virnstein, 1990), suggesting a role for climate limiting ranges. However, dispersal barriers are confounded with climate effects at 2 spatial scales. At a macrogeographic scale, the southern tip of the Florida peninsula constitutes a subtropical barrier between 2, climatologically similar temperate regions, the South Atlantic Bight and the northern Gulf of Mexico (Hedgepeth, 1953). At a mesogeographic scale along eastern Florida, the interconnected lagoons behind barrier islands present the potential for obstacles to larval dispersal (Hare and Avise, 1996), as do the oceanographic currents along the eastern Florida shelf (Hare et al., 2005).

The nonfeeding larvae of *L. panopaei* spend only 4–8 days in the plankton (at 25 C; Walker et al., 1992). Field experiments mapping new infections emanating from a point source showed a significant decrease in *L. panopaei* prevalence at distances of 1.0 and 10 m versus 0.1 m (Grosholz and Ruiz, 1995). Also, like *L. texanus*, the larvae of *L. panopaei* are intolerant of low salinity (Reisser and Forward, 1991; Walker and Clare, 1994). However, the parasite may be able to disperse better than experimental results suggest. *Loxothylacus panopaei* infections were found in the York River, Chesapeake Bay, in 1964, and as far south as North Carolina in 1983 (Hines et al., 1997). If northward range expansion was limited by physical features along the southeastern U.S. coast, these features may or may not be effective at limiting southward expansion of the introduced population as well. Since the 1980s, no data have become available on the possible spread of *L. panopaei*. To further test for limits on southward expansion of the introduced *L. panopaei* population, we measured the current geographic distribution and prevalence of this parasite along the southeastern U.S. coast during 2004 and 2005.

METHODS

Sampling

Mud crabs, 5 mm or larger, were sampled by hand from oyster beds at 11 sites in 2004 and 10 sites in 2005 along the Georgia and Florida Atlantic coasts (Table I), to measure geographic distribution and prevalence of the *L. panopaei* parasite. Genetic analyses also included parasites infecting crabs from 2 sites in the Chesapeake Bay (2002) and 1 site at Panacea, Florida (2005) in the Gulf of Mexico (Fig. 1). All crabs were preserved in 95% ethanol. Prevalence of the parasite was determined as percentage of crabs with externae. Samples of *L. texanus* were obtained in 2004 and 2005 from infected *Callinectes sapidus* in Tampa Bay and Panacea, Florida.

Genotyping

Representatives from all sites found infected in 2004 were analyzed genetically. Within the introduced range of *L. panopaei*, all samples analyzed comprised infected *Eurypanopeus depressus* hosts, except for 1 site (Queenstown, Virginia), where only parasites of *Rhithropanopeus harrisii* hosts were examined. All hosts from the indigenous range were *Panopeus* species. Approximately 20 mg of parasite tissue from the externa was used for DNA extraction with the DNeasy 96 Tissue kit (Qiagen Inc., Valencia, California); the protocol for animal tissues was used. Eluted genomic DNA was diluted 1:10. A portion of the mitochondrial cytochrome c oxidase subunit I (COI) was initially amplified with the polymerase chain reaction (PCR) with the use of the Folmer et al. (1994) primers, LCO 1490 and HCO 2198, on *L. panopaei* from St. Marys, Georgia. From this sequence, we designed more specific primers (Lxpa-L, 5′-GAGCAAGATTAATTGGAGGAGGT-3′ and Lxpa-R, 5′-GCCCCAGCTAAAACTGGTAA-3′). The PCR conditions used to amplify a COI gene fragment with the latter primers in a 20-μl reaction were: 1× *Taq* PCR buffer, 0.6 units *Taq* (both Invitrogen, Carlsbad, California), 1.0 μl DNA template, 20 μM BSA, 5 mM $MgCl_2$, 0.2 μM each primer, and 0.25 mM dNTPs. These reactions were run on a MJ Peltier PTC-255 thermocycler (MJ Research, Watertown, Massachu-

TABLE I. Locations and collection dates for infected crab samples used in genetic analysis and estimating prevalence of *Loxothylacus panopaei*; Tampa 1 and 2 sites sampled for *L. texanus* only, Panacea sampled for *L. panopaei* and *L. texanus*.

Site	Coordinates	Date in 2004	Date in 2005
Atlantic coast			
Chesapeake, Oxford	38°41′N, 076°10′W	(September 2002)	
Chesapeake, Queenstown	37°40′N, 076°29′W	(September 2002)	
Savannah	31°57.069′N, 081°05.891′W	10 July	
Sapelo	31°23′N, 081°16′W	21 July	
Brunswick	31°09.235′N, 081°34.203′W	11 July	
St. Mary	30°43.198′N, 081°32.825′W	12 July	
Jacksonville	30°23.832′N, 081°26.138′W	13 July	20 April
Camachee Harbour	29°54.970′N, 081°18.419′W		21 April
San Sebastian River	29°53.548′N, 081°19.322′W		26 April
St. Augustine Island	29°47.421′N, 081°16.181′W	19 July	
Whitney Lab	29°40.209′N, 081°12.940′W	20 July	26 April
Halifax Creek	29°24.492′N, 081°05.999′W		25 April
New Smyrna Beach	29°01.561′N, 080°55.214′W	14 July	
Edgewater, Kennedy Park	28°59.619′N, 080°54.225′W		24 April
Edgewater Landing	28°56.884′N, 080°52.504′W	13 July	
Frontenac	28°27.589′N, 080°45.679′W	14 July	
Sebastian River	27°50.071′N, 080°29.843′W		23 April
Roseland	27°50.710′N, 080°29.014′W	17 July	
Wabassoo	27°45.582′N, 080°25.045′W		21 April
Fort Pierce, Jack Island	27°29.906′N, 080°18.715′W	16 July	2 June
Fort Pierce, Jeff Island	27°28.492′N, 080°19.226′W		22 April
Gulf of Mexico			
Panacea	30°01′N, 084°23′W		January
Cedar Key 01	29°08.325′N, 083°02.063′W		16 April
Cedar Key 02	29°09.810′N, 083°01.631′W		17 April
Crystal River	28°54.672′N, 082°41.539′W		17 April
Tampa 1	27°46.261′N, 082°26.970′W	12 December	
Tampa 2	27°43.303′N, 082°44.090′W	29 June	

setts) at an initial 95 C for 5 min, then 36 3-step cycles of 95 C for 30 sec, 55 C for 1 min, 72 C for 45 sec, and, finally, 72 C for 10 min and 24 C for 2 min. A negative control (no template) was included with each set of reactions to monitor for contamination. For sequencing, template was prepared with the use of shrimp alkaline phosphatase and exonuclease, then cycle sequenced directly with each of the PCR primers in separate reactions with BigDye chemistry (Applied Biosystems, Foster City, California) and the vendor's recommended protocols. Sequences were electrophoresed on a 3100 Genetic Analyzer (Applied Biosystems).

Genetic analyses

The forward and reverse COI sequences were aligned for each individual using Sequencher (Version 4.1, GeneCodes), and automated nucleotide base calls were manually confirmed from the chromatograms. An alignment of sequences from all individuals was then constructed, and polymorphisms were double-checked for sequencing accuracy. Finally, sequence ends were trimmed to remove less-reliable base calls and make sequence lengths more uniform among individuals.

DNA polymorphism and divergence statistics were calculated with the use of DNAsp Version 4.10 (Rozas et al., 2003). Parsimony and neighbor-joining phylogenies were generated with the use of PAUP* 4.0b10 (Swofford, 2003); 1,000 bootstrap replicates were used in both cases to estimate the strength of support for each node in the tree. Neighbor-joining search was set to ''ties broken randomly'' and the Kimura 2-parameter was used to estimate genetic distance.

Comparison of the consensus COI sequence for *L. panopaei* to the GenBank sequence database with the use of nucleotide BLAST returned *Sacculina carcini* (accession number AY117692.1) as the most similar COI sequence. The *S. carcini* sequence was used as an outgroup for phylogenetic analyses.

RESULTS

In total, 1,631 crabs were collected, 620 in 2004 and 1,011 in 2005 between Savannah, Georgia, and Fort Pierce, Florida (Table II). In 2004, *L. panopaei* infections were present from Savannah, Georgia, to Jacksonville, Florida, but between Jacksonville and the endemic range limit, Cape Canaveral, only uninfected crabs were found (n = 158 from 4 sites). Infected populations in the nonindigenous range had prevalences ranging from 46 to 67%. In 2005, the parasite remained at high prevalence in Jacksonville and was found at 6 sites further south, as far as Edgewater, Florida (170 km). Prevalence was as high as 93% in recently invaded populations sampled in 2005. In both years, the mud crab populations south of Cape Canaveral had relatively low prevalence (6–16%) of (presumably native) *L. panopaei*.

Our sampling revealed a different host spectrum for *L. panopaei* infections north and south of Cape Canaveral (Table II).

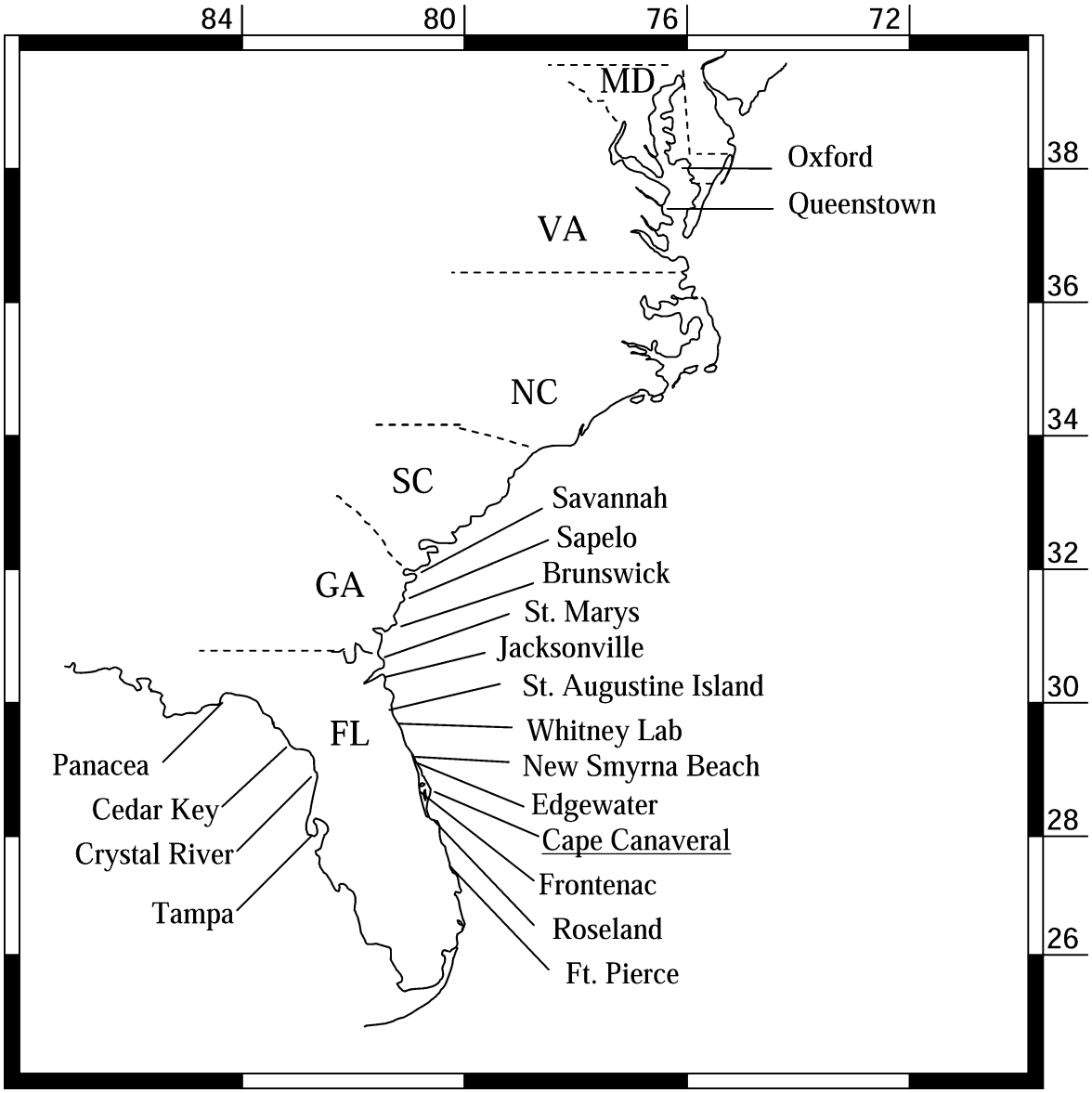

FIGURE 1. Representative sites sampled in 2004 and 2005 for estimating infection prevalence and for genetic analysis of *Loxothylacus panopaei*. MD: Maryland; VA: Virginia; NC: North Carolina; SC: South Carolina; GA: Georgia; FL: Florida.

Based on data from 2004 and 2005, the parasite was only found in *Eurypanopeus depressus* or *Rhithropanoeus harrisii* hosts in its introduced range from Chesapeake Bay down to Edgewater, Florida, whereas south of Cape Canaveral it only occurred in *Panopeus* spp. The few samples obtained from western Florida in 2004 were also all from *Panopeus* spp. infections. Collections in 2005 from the Florida west coast provided no information on host spectrum because no infections were found among 247 *Eurypanopeus depressus* and 36 *Panopeus* spp.

In total, 60 *L. panopaei* COI sequences were collected with a maximum length of 509 nucleotides. No insertions or deletions were required for alignment of the sequences and all had a continuous reading frame based on an amino acid translation inferred with the use of the *Drosophila* sp. genetic code.

To eliminate missing data, the DNA sequence alignment was trimmed at each end, making an alignment of 446 base pairs (bp). Phylogenetic analysis using parsimony and neighbor-joining methods produced identical trees with strong bootstrap support for 2 monophyletic *L. panopaei* clades (Fig. 2). Samples from sites north of Cape Canaveral grouped together with 100% bootstrap support, as did samples from south of Cape Canaveral plus the Gulf of Mexico. These patterns are based on a total of 137 variable nucleotide sites in the analyzed alignment, 49 of which were parsimony informative (occurred in 2 or more sequences). Within *L. panopaei* there were 72 variable sites, including 36 parsimony informative. Translated sequences had a total of 11 variable amino acids.

The amount of DNA polymorphism, measured as the average pairwise sequence difference (uncorrected for multiple hits), was greatest in *L. texanus* (0.0082 ± 0.0021 SD) and very low in the northern and southern *L. panopaei* clades (0.0025 ± 0.0003 and 0.0, respectively). These intraclade polymorphisms

TABLE II. Prevalence of *Loxothylacus panopaei* infections in 3 crab host genera in 2004 and 2005 at Georgia and Florida Atlantic sites plus 3 sites at Cedar Key and Crystal River, Gulf of Mexico; sites arranged from north to south (Atlantic) and west into the Gulf of Mexico, with introduced range above the double line and indigenous range below.

	Eurypanopeus depressus				*Panopeus* spp.				*Rhithropanopeus harrisii*			
	2004		2005		2004		2005		2004		2005	
	N	% Infection	N	% Infection	N	% Infection	N	% Infection	N	% Infection	N	% Infection
Savannah	6	67			6	0						
Sapelo	16	50			25	0						
Brunswick	19	63			24	0						
St. Marys	24	54			44	0						
Jacksonville	26	46	48	31	3	0	32	0				
Camachee Harbour			33	30			61	0				
San Sebastian River			8	88			1	0				
St. Augustine Island	12	0			50	0						
Whitney Lab	21	0	47	55	18	0	12	0				
Halifax Creek			28	93			0					
New Smyrna Beach	31	0			0							
Edgewater, Kennedy Park			71	58			11	0				
Edgewater Landing	22	0			4	0						
Frontenac	13	0			0				11	0		
Sebastian River			0				0				75	0
Roseland	8	0			16	6			1	0		
Wabasso			67	0			10	10				
Fort Pierce, Jack Island	86	0	52	0	134	16	56	0				
Fort Pierce, Jeff Island			105	0			8	0				
Cedar Key 1			101	0			11	0				
Cedar Key 2			39	0			8	0				
Crystal River			107	0			17	0			3	0
Sum	284		706		324		227		12		78	

were all at synonymous sites. The average number of substitutions per site between the northern and southern *L. panopaei* clades, after correction for ancestral polymorphism (Nei and Li, 1979) and multiple hits (Jukes and Cantor, 1969), was 0.077 ± 0.004. The same divergence measure calculated between *L. texanus* and the northern and southern clades was 0.129 ± 0.014 and 0.143 ± 0.023, respectively.

DISCUSSION

Range extension

The high rate of southward population expansion and the high parasite prevalence at the leading edge provide unexpected results from this study. The southward extension of the nonindigenous *L. panopaei* population from Bogue Sound, North Carolina, in 1983 (Hines et al., 1997) to Jacksonville, Florida, in 2004 (this study), amounts to an advance of 700 km in 21 yr, or 33.3 km/yr. In contrast, the southward extension of visibly parasitized crabs from 2004 to 2005 indicates a maximum rate of advance of 170 km/yr. Both rates fall into the range of previously estimated average extension rates for nonindigenous marine invertebrates (16–235 km/yr; Kinlan and Gaines, 2003). The lower estimate is also consistent with the conclusion of Hines et al. (1997), based on the rate of spread and scale of prevalence patterns, that dispersal of *L. panopaei* operates on a scale of tens of kilometers per generation.

However, it is difficult to interpret the present data on rate of *L. panopaei* range expansion in terms of an annual average of southward range shift. First, expansion rates of nonindigenous species are known to vary widely from year to year for many reasons (Grosholz, 1996; Shanks et al., 2003). Prevalence of *L. panopaei* in host populations near the expanding front also seems to vary considerably from year to year, complicating the assessment of changes in the parasites' range distribution. For example, the 47.4% infection prevalence observed at the North Carolina front in 1983 dropped to 0 infections in 1986 (Hines et al., 1997). Finally, it is still unknown which mechanism of dispersal *L. panopaei* uses in the field, which makes any assessment of the scale of its dispersal potential problematic. The possibility that southward currents may have facilitated the southward expansion of *L. panopaei* is in contrast to the rapid northward expansion in this region of *Perna perna*, the invasive green mussel. This is interesting because the range expansion of the mussels is also thought to have been dependent upon larval transport by inshore currents (Hicks and Tunnell, 1995).

The high parasite prevalence documented here for recently invaded host communities is similar to results described by Hines et al. (1997). Both studies measured prevalence in crab populations that had been infected for no more than 22 yr. Although we found prevalences ranging between 30 and 93% in Georgia and Florida, Hines et al. (1997) reported prevalences between 47 and 83% in Virginia and North Carolina, respectively, indicating that epidemic infections occurred in recently invaded areas. Hines et al. (1997) also showed that prevalence differed significantly between years, as well as between nearby sites. In the Chesapeake Bay, for example, they found a prevalence of over 90% at 1 site, whereas at all other Chesapeake

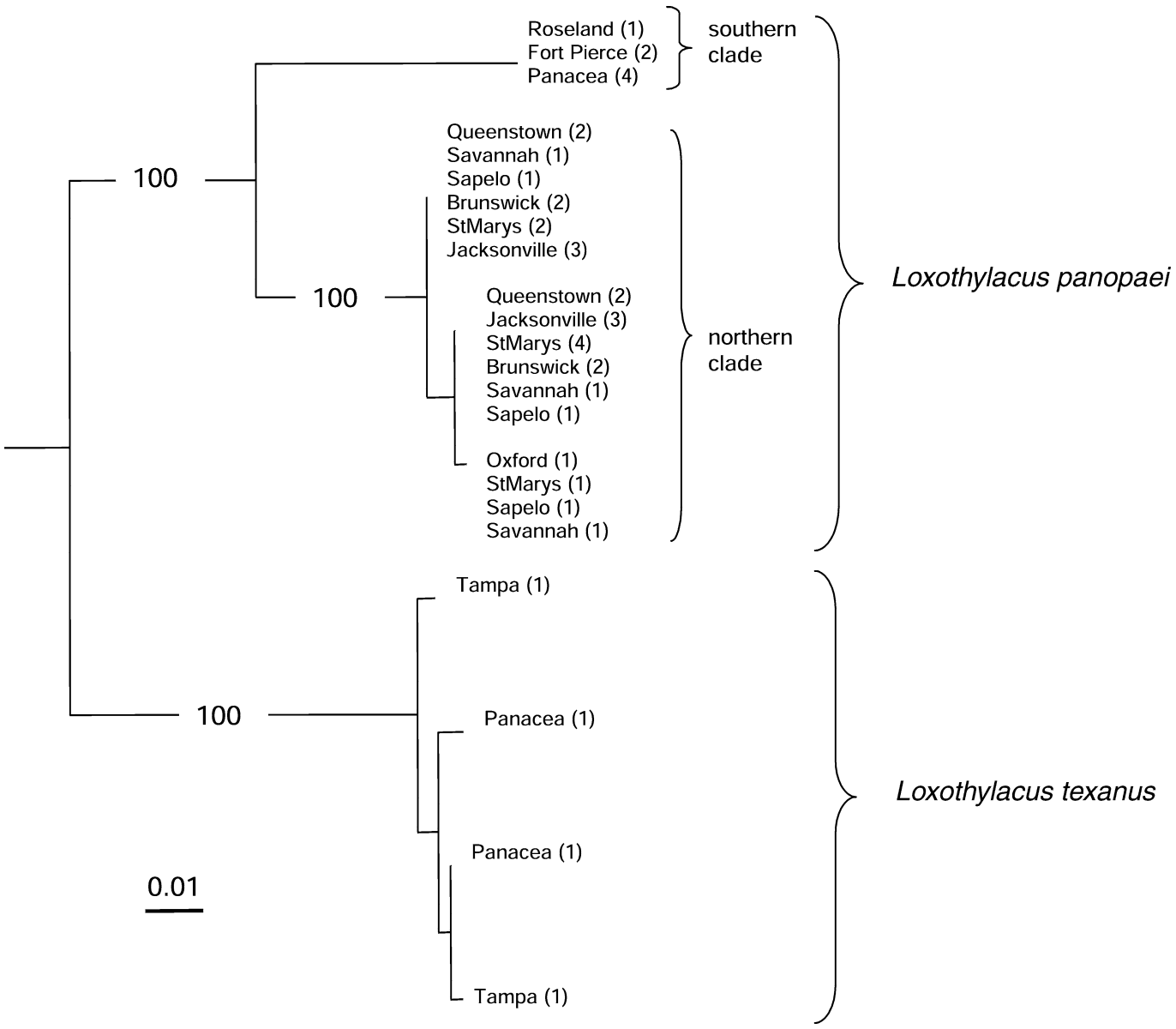

FIGURE 2. Outgroup rooted neighbor joining phylogram of COI sequences from *Loxothylacus panopaei* and *L. texanus*, with *Sacculina carcini* used as the outgroup. Bootstrap confidence values greater than 70% are shown on branches. The scale bar indicates nucleotide substitutions per site. Identical haplotypes from *L. panopaei* are found at different sites; numbers of specimens with identical haplotypes from the same site are indicated in brackets. Northern clade *L. panopaei* from *Eurypanopeus depressus* and *Rhithropanopeus harrisii* (Queenstown only) hosts; southern clade specimens from *Panopeus* species.

sites, it was lower than 13% in the same year. This indicated highly fluctuating population dynamics in the northern nonindigenous range of this parasite. In contrast, in the southern native range, the maximum prevalence found in *Panopeus* spp. hosts in this study (16%) and by Hines et al. (1997) (9.3%) was much lower than in the nonindigenous range. Because geographic and genetic structure are confounded in these parasite populations, it is impossible to attribute high prevalence to a single cause; the north–south difference in prevalence may stem from genetic differences between the northern and the southern clades of parasite, irrespective of invasion dynamics, or result from the altered demographics of a parasite invading naïve host populations.

Genetic and host variation

The phylogenetic resolution of 2 exclusive *L. panopaei* clades is extremely robust based on mitochondrial COI sequences and provides a strong rejection of Florida as the source population. The COI clades define groups of individuals with nonoverlapping geographic ranges and distinct host spectra, 2 important biological attributes that typically constitute species-level distinction. Without examining additional characters from independent loci or morphology, however, it is premature to conclude that these clades represent different *Loxothylacus* species. Two alternative hypotheses deserve consideration, i.e., differences are due to evolutionary changes during and after in-

vasion. Alternatively, genetic and phenotypic population structure occurs between eastern and western Gulf of Mexico *L. panopaei* populations, and the latter (not included in this study) was the source for the Chesapeake introduction.

Differences between introduced and indigenous populations could be caused by founder effects, genetic drift, and/or selection, during the invasion and establishment of *L. panopaei* in Chesapeake Bay (e.g., Frankham and Ralls, 1998; Lee, 2002; Allendorf and Lundquist, 2003). However, founder individuals draw variation from the source population and would only show reciprocal monophyly with an average 8% interclade divergence if the source population(s) were severely undersampled. Both the indigenous and nonindigenous clades showed similarly low levels of COI variation in Florida compared to 4 outgroup *L. texanus* specimens (despite unequal sample sizes and source areas that would bias the comparison in the other direction). Therefore, the founder-effect hypothesis requires that additional unsampled genetic variation be present in the Gulf of Mexico. *Loxothylacus panopaei* infecting *Eurypanopeus depressus* or *Rhithropanopeus harrisii* were found in small numbers in Apalachicola, western Florida, by Hines et al. (1997), and in Louisiana and Texas by Reinhard and Reischman (1958). Further genetic sampling from the western Gulf is necessary to determine how much of the Chesapeake–Florida contrast is due to founder effects versus Gulf of Mexico population structure. In either case, the contrast in sequence diversity between *L. panopaei* and *L. texanus* in the eastern Gulf of Mexico is striking and might stem from biological attributes that determine different effective population sizes.

A 3rd possibility is that the introduced Chesapeake population is a *Loxothylacus* sp. from a different part of the world. Unfortunately, no phylogenetic work has been done on this genus, and the biogeography and host-use variation within the genus suggests multiple candidates. In addition to *L. panopaei*, 7 *Loxothylacus* species primarily infect xanthid crabs (from 1 to 5 xanthid host species per parasite species) and these occur in eastern Asia, the Indian Ocean, and the Red Sea (Boschma, 1955).

Northern range limit of *L. panopaei*

Indigenous *L. panopaei* from southeast Florida and the eastern Gulf, as defined here by the southern genetic clade, appear to parasitize species of *Panopeus* primarily. *Panopeus lacustris*, the most common host in southeast Florida, has its natural northern range limit at Cape Canaveral. In contrast, the preferred host for nonindigenous parasites north of Cape Canaveral, *Eurypanopeus depressus*, is also very abundant in southeast Florida, but very rarely infected (Table II; Hines et al., 1997). These results indicate differences in host specificity between the 2 genetically different *L. panopaei* populations north and south of Cape Canaveral. Consequently, the northern range limit of the indigenous population of *L. panopaei* at Cape Canaveral may be determined indirectly by the parasite's preference for the geographically restricted host *P. lacustris*. Although a northern range expansion of *L. panopaei* across Cape Canaveral may be limited by host availability, a southward range expansion of the northern population across Cape Canaveral appears possible from this aspect.

Host range limits, potential dispersal barriers, and a climatological gradient are all spatially confounded at Cape Canaveral, so tearing apart their impacts on range limits will be challenging. As the range expansion of introduced *L. panopaei* brings this population closer to a major biogeographic boundary and nominally conspecific populations, this natural experiment provides valuable opportunities to evaluate larval dispersal barriers at Cape Canaveral, host spectrum of this parasite, and contrast invasion dynamics in communities with naïve versus coevolved hosts.

ACKNOWLEDGMENTS

We wish to thank Daniel Beissel, Maria Murray, Dale Bishop, and Sherry Reed for help on field trips and Kristina Cammen for dissecting crabs. Specimens of *L. texanus* were kindly provided by A. McMillan-Jackson, S. Schmitt, and their associates at the Florida Marine Research Institute. Chesapeake specimens of *L. panopaei* were provided by M. Torchin. We are grateful to Darryl Felder for species identifications of some xanthid crabs. This work was supported by University of Maryland funds to M.P.H.

LITERATURE CITED

ALLENDORF, F.W., AND L. L. LUNDQUIST. 2003. Introduction: Population biology, evolution and control of invasive species. Conservation Biology **171:** 24–30.

ALVAREZ, F., A. H. HINES, AND M. L. REAKA-KUDLA. 1995. The effects of parasitism by the barnacle *Loxothylacus panopaei* (Gissler) (Cirripedia, Rhizocephala) on growth and survival of the host crab *Rhithropanopeus harrisii* (Gould) (Brachyura, Xanthidae). Journal of Experimental Marine Biology and Ecology **192:** 221–232.

BASTROP, R., K. JÜRSS, AND C. STURMBAUER. 1998. Cryptic species in a marine polychaete and their independent introduction from North America to Europe. Molecular Biology and Evolution **152:** 97–103.

BLANK, M., R. BASTROP, M. RÖHNER, AND K. JÜRSS. 2004. Effect of salinity on spatial distribution and cell volume regulation in two sibling species of Marenzelleria Polychaeta: Spionidae. Marine Ecology Progress Series **271:** 193–205.

BOONE, E., A. A. BOETTCHER, T. D. SHERMAN, AND J. J. O'BRIEN. 2004. What constrains the geographic and host range of the rhizocephalan *Loxothylacus texanus* in the wild? Journal of Experimental Marine Biology and Ecology **309:** 129–139.

BOSCHMA, H. 1955. The described species of the family *Sacculinidae*. Zoologische Verhandelingen **27:** 1–76.

———. 1972. On the occurrence of *Carcinus maenas* (Linnaeus) and its parasite *Sacculina carcini* Thompson in Burma, with notes on the transport of crabs to new localities. Zoologische Mededelingen (Leiden) **47:** 145–155.

BRIGGS, J. C. 1974. Marine zoogeography. McGraw-Hill, New York, New York, 475 p.

BURRESON, E. M., N. A. STOKES, AND C. S. FRIEDMAN. 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. Journal of Aquatic Animal Health **12:** 1–8.

CARLTON, J. T. 1996. Biological invasions and cryptogenic species. Ecology **77:** 1653–1655.

DAVIES, N., F. X. VILLABLANCA, AND G. K. RODERICK. 1999. Determining the sources of individuals in recently founded populations: Multilocus genotyping in non-equilibrium genetics. Trends in Ecology and Evolution **14:** 17–21.

FOLMER, O., M. BLACK, W. HOEN, R. LUTZ, AND R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metozoan invertebrates. Molecular Marine Biology and Biotechnology **3:** 294–299.

FRANKHAM, R., AND K. RALLS. 1998. Conservation biology: Inbreeding leads to extinction. Nature **392:** 441.

GASTON, K. J. 2003. The structure and dynamics of geographic ranges. Oxford University Press, Oxford, U.K., 304 p.

GELLER, J. B., E. D. WALTON, E. D. GROSHOLZ, AND G. M. RUIZ. 1997. Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. Molecular Ecology **6:** 901–906.

GISSLER, C. F. 1884. The crab parasite, *Sacculina*. American Naturalist **18:** 226–229.

GOZLAN, R. E., S. ST-HILAIRE, S. W. FEIST, P. MARTIN, AND M. L. KENT. 2005. Disease threat to European fish. Nature **435:** 1046.

GROSHOLZ, E. D. 1996. Contrasting rates of spread for introduced species in terrestrial and marine systems. Ecology **77:** 1680–1686.

———, AND G. M. RUIZ. 1995. Does spatial heterogeneity and genetic variation in populations of the xanthid crab *Rhithropanopeus harrisii* Gould influence the prevalence of an introduced parasitic castrator? Journal of Experimental Marine Biology and Ecology **187:** 129–145.

GUILLORY, V., H. M. PERRY, P. STEELE, T. WAGNER, P. HAMMERSCHMIDT, S. HEATH, AND C. MOSS. 1998. The Gulf of Mexico blue crab fishery: Historical trends, status, management, and recommendations. Journal of Shellfish Research **172:** 395–404.

HARE, M. P., AND J. C. AVISE. 1996. Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). Evolution **50:** 2305–2315.

———, C. GUENTHER, AND W. F. FAGAN. 2005. Nonrandom larval dispersal can steepen marine clines. Evolution **59:** 2509–2517.

HEDGEPETH, J. W. 1953. An introduction to the zoogeography of the northwestern Gulf with reference to the invertebrate fauna. Publications of the Institute of Marine Science, University of Texas **107:** 107–224.

HICKS, W., AND J. W. J. TUNNELL. 1995. Ecological notes and patterns of dispersal in the recently introduced mussel, *Perna perna* (Linné, 1758), in the Gulf of Mexico. American Malacological Bulletin **11:** 203–206.

HINES, A. H., F. ALVAREZ, AND S. A. REED. 1997. Introduced and native populations of a marine parasitic castrator: Variation in prevalence of the rhizocephalan *Loxothylacus panopaei* in xanthid crabs. Bulletin of Marine Science **612:** 197–214.

HØEG, J. T., AND J. LÜTZEN. 1995. Life cycle and reproduction in the Cirripedia Rhizocephala. Oceanography and Marine Biology Annual Review **33:** 427–485.

HOPPE, K. N. 2002. *Teredo navalis*—The cryptogenic shipworm. *In* Invasive aquatic species in Europe. Distribution, impacts and management, E. Leppakoski, S. Gollasch, and S. Olenin (eds.). Kluwer Academic, Dordrecht, The Netherlands, p. 116–120.

HUSPENI, T. C. 2000. A molecular genetic analysis of host specificity, continental geography, and recruitment dynamics of a larval trematode in a salt marsh snail. Ph.D. Dissertation. University of California, Santa Barbara, California, 145 p.

JOUSSON, O., J. PAWLOWSKI, L. ZANINETTI, A. MEINESZ, AND C. BOUDOURESQUE. 1998. Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. Marine Ecology Progress Series **172:** 275–280.

JUKES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. *In* Mammalian protein metabolism, H. N. Munro (ed.). Academic Press, New York, New York, p. 21–132.

KINLAN, B. P., AND S. D. GAINES. 2003. Propagule dispersal in marine and terrestrial environments: A community perspective. Ecology **84:** 2007–2020.

KURIS, A. M. 1974. Trophic interactions: Similarity of parasitic castrators to parasitoids. Quarterly Review of Biology **49:** 129–148.

LEE, C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution **178:** 386–391.

MCCALLUM, H., D. HARVELL, AND A. DOBSON. 2003. Rates of spread of marine pathogens. Ecology Letters **6:** 1062–1067.

NEI, M., AND W.-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences USA **76:** 5269–5273.

NOAA WEB SITE. http://www.st.nmfs.gov/st1/commercial/landings/annual_landings.html.

PEARSE, A. S., AND L. G. WILLIAMS. 1951. The biota of the reefs off the Carolinas. Journal of the Elisha Mitchell Science Society **67:** 133–161.

PIMM, S. L. 1991. The balance of nature? Ecological issues in the conservation of species and communities. University of Chicago Press, Chicago, Illinois, 434 p.

REINHARD, E. G., AND P. G. REISCHMAN. 1958. Variation in *Loxothylacus panopaei* (Gissler), a common sacculinid parasite of mud crabs, with the description of *Loxothylacus perarmatus* n. sp. Journal of Parasitology **44:** 93–97.

REISSER, C. E., AND R. B. FORWARD. 1991. Effect of salinity on osmoregulation and survival of a rhizocephalan parasite, *Loxothylacus panopaei*, and its crab host, *Rhithropanopeus harrisii*. Estuaries **14:** 102–106.

ROZAS, J., J. C. SÁNCHEZ-DELBARRIO, X. MESSEGUER, AND R. ROZAS. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19:** 2496–2497.

SHANKS, A. L., B. A. GRANTHAM, AND M. H. CARR. 2003. Propagule dispersal distance and the size and spacing of marine reserves. Ecological Applications **13:** 159–169.

SHIELDS, J. D., AND R. M. OVERSTREET. 2003. Diseases, parasites and other symbionts. *In* The blue crab, *Callinectes sapidus*, V. S. Kennedy and L. E. Cronin (eds.). Maryland Sea Grant College, University of Maryland, College Park, Maryland, p. 223–339.

SIMBERLOFF, D., I. M. PARKER, AND P. N. WINDLE. 2005. Introduced species policy, management, and future research needs. Frontiers in Ecology and the Environment **3:** 12–20.

STREFTARIS, N., A. ZENETOS, AND E. PAPATHANASSIOU. 2005. Globalisation in marine ecosystems: The story of non-indigenous marine species across European seas. Oceanography and Marine Biology Annual Review **43:** 419–453.

SWOFFORD, D. L. 2003. PAUP*: Phylogenetic analysis using parsimony* and other methods, Version 4.0b 10 Sinauer Associates, Sunderland, Massachusetts.

TINDLE, S., E. BOONE, J. O'BRIEN, AND A. BOETTCHER. 2004. Effects of salinity on larval stages of the rhizocephalan barnacle *Loxothylacus texanus*: Survival and metamorphosis in response to the host, *Callinectes sapidus*. Journal of Experimental Marine Biology and Ecology **302:** 165–176.

TORCHIN, M., K. D. LAFFERTY, AND A. M. KURIS. 2002. Parasites and marine invasions. Parasitology **124:** 137–151.

TSUTSUI, N. D., A. V. SUAREZ, D. A. HOLWAY, AND T. J. CASE. 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences USA **97:** 5948–5953.

VAN ENGEL, W. A., W. A. DILLON, D. ZWERNER, AND D. ELDRIDGE. 1966. *Loxothylacus panopaei* Cirripedia, Sacculinidae, an introduced parasite on a xanthid crab in Chesapeake Bay, U.S.A. Crustaceana **10:** 111–112.

VIRNSTEIN, R. W. 1990. The large spatial and temporal biological variability of Indian River Lagoon. Florida Scientist **53:** 249–256.

WALKER, G., AND A. S. CLARE. 1994. The effect of salinity on the development of *Loxothylacus panopaei* larvae (Crustacea, Cirripedia, Rhizocephala). Estuaries **17:** 276–282.

———, ———, D. RITTSCHOF, AND D. MENSCHING. 1992. Aspects of the life-cycle of *Loxothylacus panopaei* (Gissler), a sacculinid parasite of the mud crab *Rhithropanopeus harrisii* (Gould)—A laboratory study. Journal of Experimental Marine Biology and Ecology **157:** 181–193.