Appendix A: Crab Identification

Methods Development

Identifying early stage crabs was a crucial yet challenging part of this research. Crucial, because it was necessary to differentiate between the different species of crabs collected through the fieldwork in order to assess their patterns of recruitment, as part of answering the research questions. Challenging, because there was not an established resource for identifying the crabs at the stages and in the locations of interest, so it was necessary to develop the approach for identification. Also, it was difficult to find descriptions of early stage crabs because while references to zoea larvae and adults were not uncommon, there were few descriptions of megalopae and even fewer descriptions of juveniles. It was often impossible to determine from the title or even the abstract of an article whether it would contain a description of an early stage crab, and the full text had to be obtained in order to determine if the article might contain useful information. Additionally, articles that might contain descriptions of early stage crabs were often in obscure or old journals, and were difficult and time consuming to obtain.

Early stage crabs were the target species because the objectives of the fieldwork were to assess patterns of recruitment, following up on lab studies that indicated enhanced molting of *H. sanguineus* larvae in the presence of materials used in aquaculture and with physical complexity (O'Connor, unpub.). The term early stage is used here to denote crabs in the megalopal stage, which is the last larval stage, through the juvenile stages. Recruitment occurs when megalopae, which have settled onto suitable habitat, molt to the first juvenile stage. Crabs passing through the first few molts are considered recruits. Recruits are small, approximately 0.5mm to 10mm in carapace width (CW), and difficult to find on natural substrate, thus recruits were collected on

materials secured in the field, called collectors. These collectors could be brought into the lab, which made it easier to remove and examine the tiny crabs.

While numbers of crabs per collector could be established at the point of removal from the collectors, in order to distinguish the patterns of recruitment of *H. sanguineus* from other crab species, the individual recruits needed to be identified as precisely as possible. Once it was decided to identify the recruits collected, the question became how to identify them.

To determine how to identify the crabs, first steps involved seeking resources such as identification keys, and people who had done similar identification work. Database searches and discussions with researchers were conducted to determine what resources were available. A larval key was obtained from Dr. Stanley Cobb at the University of Connecticut, which included information about megalopae. The information had been compiled by his former student Niels Hobbs. Other researchers who work with early stage crabs were contacted, as well as researchers involved in research on crabs in general, especially in southeastern New England, but also all along the east coast of the United States, and elsewhere. These researchers were found based on citations in papers, associations with institutions such as the Smithsonian, and suggestions by other researchers. Discussions and searches resulted in the discovery of a general lack of specific and comparative information.

Since there was not a complete key to early juvenile crab species of southeastern New England, information needed to be assembled on the various possible species that could have been collected, and a first step in this process was determining which species were likely possibilities. This was based on presence of adults in both the general geographic area - New England - and the general habitat - intertidal to shallow subtidal zones. This information was collected through books (especially Williams, 1984), articles, and interviews with researchers

and people involved in shellfish aquaculture. Some less likely crab species were also considered, such as the Mitten crab, which is not established in southeastern New England but may become established since it is a problematic invader in other regions.

Due to the lack of an established method for identifying early stage crabs in southeastern New England, major general methods of identification were evaluated for use in the present study. These methods include identification based on morphological, genetic, and behavioral traits. Methods based on genetics were ruled out due to limitation of resources. Methods based on behavior were ruled out based on lack of background information. Some information was available on morphology, including morphometrics, and thus identifications were based on morphological traits.

Then, the morphological information on each possible species was needed. Morphological descriptions were sought, and in particular, photos and figures. Information was sought on both megalopae and juveniles. Though rare, specific information at different juvenile stages was of particular interest, since characteristics can change significantly between stages. Database searches included the Internet, Zoological Record, JSTOR, ASFA, and others. Articles of interest were identified also through library shelves, consultation with the UMass Dartmouth science librarian, literature cited sections of other articles, and discussions with researchers. Articles were obtained through the UMass Dartmouth Library, Interlibrary Loan, and contacting researchers directly.

While some comparative sources were found for megalopae (Cobb key, field guide), hardly any information was found for juveniles (Felder et al. 1985), so it was necessary to examine individual articles on development and identification of single or small groups of species that might mention juvenile stages. Even for these articles, much more information was

available on megalopae, as the last stage of larval development, than juveniles. Information was limited but it was possible to acquire at least some information on most species, and more extensive information on some species, including morphometrics. For gaps in information, in some cases it was possible to look at related species found in other locations.

Morphological characteristics of the likely species were compiled and compared. Photographs and figures, and descriptions, for each species were collected together and compared and synthesized. If discrepancies were found, both sets of information were kept because with so little information it was not necessarily clear which was correct or if the differences might be indication of variation. Species descriptions were compared, and similarities and differences in characteristics that could be used in identification were noted. The most focus was placed on information on characteristics that could be seen with a dissecting scope, externally, that were quantifiable and prominent, and that there existed information for multiple species. Since *Carcinus maenas, Hemigrapsus sanguineus* and members of the family Xanthidae were likely species, particular focus was placed on compiling information on these species. It was decided to lump some species (Xanthidae, primarily) by family due to sparse information and/or minor differences at the species level.

The next step was comparing the characteristics of the crabs collected to the possible species they could be. Morphological information from the collected crabs was gathered through initial observations by naked eye and dissecting scope at the time of removal from the collector material, microscope ditigal photography, and reference back to the original specimen as necessary. The microscope digital photography was particularly helpful because the dissecting scope provided adequate magnification and the photographs could then be compared to the figures and descriptions. Photographs were taken through a Leica MZ9.5 dissecting microscope

with a Leica DFC490 digital camera. Crabs were placed on slides with concave centers. Measures were taken to facilitate observations of live crabs based on previous work with decapods (Spence et al., *in review*). While it is best to observe the crabs alive, after initial observations and photos, the crabs were frozen to preserve them for future reference, which is better than chemicals for maintaining color patterns.

Measurements were taken from the photographs using ImagePro Plus. Measurements had to be calibrated with the same slides used for the photographs. Magnification was noted in the saved photograph name to achieve an accurately calibrated measurement. Carapace length and width were the chosen quantities to measure for all crabs. The carapace was chosen because it is the largest feature, and is clearly visible and present for all the crabs (unlike pereiopods), and is structurally strong so that it holds up well. Also, information about carapace shape and size is a common description and given in several sources for comparison. Carapace width was measured between the tips of the last set of anterolateral spines, and carapace length was measured between the midpoints of the frontal and posterior margins.

Morphological distinctions and comparisons could be made directly from photos. This had many advantages, including protecting the original specimen, which could be referred to if necessary. Measurements were a helpful guide, particularly when they could be used to determine how developed certain characteristics should be at the various possibilities of stage of that crab. The process of comparison was iterative, and further research and examination was conducted as necessary.

The classification down to family or species was determined based on comparisons of characteristics. Identifications were made based on multiple matches of characteristics, and expert opinion. Some characteristics were found to be more useful than others in practice (for

present study, and predicted for other field-based studies), such as shape and number of anterolateral teeth, shape of the frontal margin, and chela coloration.

The results of the crab identification portion of this research have broader applications. In addition to supporting the present research goals, this morphological work will support similar research that follows or builds upon the present study. It will facilitate tracking and monitoring crab species before adulthood, and making predictions or assessments at the megalopal or juvenile stages, which is important because early stage crabs play different ecological roles than adults, and help to determine the extent and distribution of adult populations. Identification of early stage invasive species, such as *Hemigrapsus sanguineus*, can help in early detection. Accurate and precise identification of research subjects is important in general as a tool for research at any scale.

Appendix A continued:
Identification Guide to Early Stage Intertidal and Shallow-Subtidal Crabs in Southeastern New England

Information that was collected and used to aid in identification of crabs for the present study is included here, including notes and tables on morphological characteristics, and an annotated select bibliography.

The focus of identification was on juvenile stages, however some information for larval and adult crabs was also collected and is included.

Particular focus was placed on *Hemigrapsus sanguineus*, *Carcinus maenas*, and Xanthid crabs because these were the most common and likely crabs to collect in the investigation.

More information was available on *C. maenas* and Xanthidae than on *H. sanguineus*.

More information was available on larval and megalopal stages than juvenile stages.

For juvenile crabs, common useful morphological characteristics in identification were:

Xanthidae – broad chela with black tips
H. sanguineus – square carapace, bilobed frontal margin, banded coloration
C. maenas – slightly protruding frontal margin

A commonly used method for adult identification – the number of anterolateral spines – is useful in identification of juveniles, but only as a general guide because the number and shape of these spines changes as crabs progress through the juvenile stages.

The following notes, tables, and annotated bibliography are included as a further guide to characteristics useful in identification and resources for further information.

NOTES ON IDENTIFYING MEGALOPAL, JUVENILE, AND ADULT CRABS

General Sources: (1) Johnson and Allen, 2005 (2) A. B. Williams, 1984

(Other sources are cited individually.)

Identifying Characteristics by family:

Grapsidae (shore marsh and talon crabs) –

Hemigrapsus sanguineus (Asian shore crab) –

Adults with 3 teeth and square carapace

Megalopae rectangular/elongate with cleft on anterior margin (2)

Juveniles – carapace square, bilobed frontal margin (Muraoka, 1971)

Portunidae (swimming crabs) -

Carcinus maenas (shore crab, green crab) –

Adults 5 teeth, distal articles 5th legs not paddlelike (1)

Megalopae with short pointed rostrum (2)

Junveniles – In first young crab stage, five teeth are present however the first, third, and fifth are sharper and more prominent [so be careful in consideration with the three teeth of H. sanguineus!]; the total length of the carapace is approx. equal to the width between the posterior pair of teeth; for shape of progressive juvenile stages see drawings in figure 20, p. 19 (Shen, 1935) and also photos in figure 1 p. 338 (Hogarth, 1978). Stage 1 frontal region is long, very broad, and obliquely deflexed – frontal margin with triangular median prominence. All stages frontal margin with medial prominence (?) (Shen, p.19).

Callinectes sapidus (Blue Crab) –

Adults 9 teeth, cheliped carpus w/out mesiodistal spine (1)

Megalopae w/ small spines from posterior margin carapace (2)

Ovalipes spp. (Lady crab) –

Adults 5 teeth, distal articles 5th legs paddlelike (1)

Megalopae with central points on sides of carapace (2)

Xanthidae (mud crabs: panopeids) –

Adult teeth vary

Megalopae - some megalopa with square shape - small rostral horns (Dyspanopeus texanus), or blunt rostrum (Eurypanopeus depressus) (2)

Juveniles - First crab stage, three to four teeth; drawings p. 557 (Felder and Truesdale, 1984). Stage two, four teeth, p.563. Stage three, four teeth present with a fifth tooth (between 1 and 2) beginning to bulge. Stage five, 5 teeth present (2^{nd} inconspicuous); see figures of all stages (Felder and Truesdale, 1984). Frontal margin slightly bilobed,central medial depression (FandT, p. 565 e.g.)

Majidae (spider crabs) –

Adults with unique carapace shape, long chelipeds, hooked hairs, some with no teeth (1)

Megalopae with very irregular surface on carapace (2)

Cancridae (rock and jonah crabs) –

Adults with 9 quadrangular/pentagonal lobed teeth (1)

Megalopae with strong dorsal spine and pointed rostrum (2)

TABLES OF MORPHOLOGICAL CHARACTERISTICS

Carapace sizes for juvenile stages of *Hemigrapsus sanguineus* (Asian shore crab), Carcinus maenas (Green crab), and *Panopeus herbsteii* and other Panopeid species (Mud crab). CW=carapace width; CL=carapace length. (Muraoka, 1971; Shen 1935; Hebling 1982; Martin et al 1984)

Family	Species	Stage	CW (mm)	CL (mm)	CL/CW
Grapsidae	H. sanguineus	all	2-10		nearly 1
Portunidae	C. maenas	1	1.86	1.92	1.03
Portunidae	C. maenas	2	2.6	2.38	0.92
Portunidae	C. maenas	3	3.52	3.1	0.88
Portunidae	C. maenas	4	4.51	3.83	0.85
Portunidae	C. maenas	5	5.82	4.9	0.84
Portunidae	C. maenas	6	7.64	6.22	0.81
Portunidae	C. maenas	7	10.2	8.1	0.79
Portunidae	C. maenas	8	13.12	10.6	0.81
Portunidae	C. maenas	9	18.36	14.37	0.78
Xanthidae	P. herbstii	1	1.21	1.13	0.93
Xanthidae	P. herbstii	2	1.67	1.43	0.86
Xanthidae	P. herbstii	3	2.21	1.84	0.83
Xanthidae	P. herbstii	4	2.7	2.2	0.81
Xanthidae	P. herbstii	5	3.21	2.5	0.78
Xanthidae	P. herbstii	6	4.39	3.35	0.76
Xanthidae	P. herbstii	7	5.69	4.3	0.76
Xanthidae	P. herbstii	8	6.13	4.49	0.73
Xanthidae	Panopeid spp	1	~1mm		
Xanthidae	Panopeid spp	2	1-1.5		
Xanthidae	Panopeid spp	3	<~2mm		less than 1
Xanthidae	Panopeid spp	5	~2mm+		less than 1

Morphological characteristics of juvenile stages of *Hemigrapsus sanguineus* (Asian shore crab), Carcinus maenas (Green crab), and *Panopeus herbsteii* and other Panopeid species (Mud crab). FM=frontal margin; FMS=frontal margin spines or lobes; ALS=anterolateral spines. (Muraoka, 1971; Shen 1935; Hebling 1982; Martin et al 1984)

Family	Species	Stage	FM shape	FM midline	#FMS	#ALS
Grapsidae	H. sanguineus	all	"flat" slightly bilobed	depression	2	3
Portunidae	C. maenas	1	long, broad, obliquely deflexed	prominence	3	5
Portunidae	C. maenas	2	broader, becoming trilobed	prominence	3	5
Portunidae	C. maenas	3	broader, becoming trilobed	prominence	3	5
Portunidae	C. maenas	4	somewhat trilobed	prominence	3	5
Portunidae	C. maenas	5	"flat" (slightly trilobed)	prominence	3	5
Portunidae	C. maenas	6	"flat" (slightly trilobed)	prominence	3	5
Portunidae	C. maenas	7	"flat" (slightly trilobed)	prominence	3	5
Portunidae	C. maenas	8	"flat" (slightly trilobed)	prominence	3	5
Portunidae	C. maenas	9	"flat" (slightly trilobed)	prominence	3	5
Xanthidae	P. herbstii	1	Bilobed	depression	2	3
Xanthidae	P. herbstii	2	Bilobed	depression	2	3
Xanthidae	P. herbstii	3	"flat" (very slightly bilobed)	depression	2	4
Xanthidae	P. herbstii	4	"flat" (very slightly bilobed)	depression	2	4.5
Xanthidae	P. herbstii	5	"flat" (very slightly bilobed)	depression	2	4.5
Xanthidae	P. herbstii	6	"flat" (very slightly bilobed)	depression	2	5
Xanthidae	P. herbstii	7	"flat" (very slightly bilobed)	depression	2	5
Xanthidae	P. herbstii	8	"flat" (very slightly bilobed)	depression	2	5
Xanthidae	Panopeid spp	1	"flat" (slightly bilobed)	depression		3 to 4
Xanthidae	Panopeid spp	2	"flat" (slightly bilobed)	depression		~4
Xanthidae	Panopeid spp	3	"flat" (slightly bilobed)	depression		4
Xanthidae	Panopeid spp	5	"flat" (slightly bilobed)	depression		5

Excerpt from Spence, H. R., O'Connor, N. J., & Chen, C. (2008). *Recruitment of the Invaders: Factors Affecting the Distribution of Early Stage Hemigrapsus Sanguineus and Other Crabs in Southeastern Massachusetts with Implications for Shellfish Aquaculture: A Thesis in Marine Biology* (University of Massachusetts Dartmouth).

Appendix A continued: Annotated Bibliography

Costlow, J.D. and C.G. Bookhout. 1961. The larval stages of *Panopeus herbstii* Milne-Edwards reared in the laboratory. Journal of the Elisha Mitchell Scientific Society 77:33-42.

Describes and provides figures for zoeal through megalopal stages of *P. herbstii*.

Costlow, J.D. and C.G. Bookhout. 1966. The larval development of *Ovalipes ocellatus* (Herbst) under laboratory conditions. Journal of the Elisha Mitchell Scientific Society 82(2):160-171.

Describes larval stages of *O. ocellatus*, Lady crab, through megalopal.

Felder, D.L., J.W. Martin, and J.W. Goy. 1985. Patterns in early postlarval development of decapods. *In* Crustacean Issues II. Larval Growth. Balkema, Boston.

Describes difficulties with identifying juvenile crabs, and includes some figures of early stage crabs at different stages.

Haefner, P.A. 1977. Aspects of the biology of the jonah crab, *Cancer borealis* Stimpson, 1859 in the mid-Atlantic Bight. Journal of Natural History 11:303-320.

Discusses aspects of immature *C. borealis* and *C. irroratus*. No figures.

Hart, J.F.L. 1935. The larval development of British Columbia Brachyura; 1. Xanthidae, Pinnotheridae (in part) and Grapsidae. Canadian Journal of Research 12(4).

Includes megalopa and first young crab stage for *Hemigrapsus nudus*, which bear similarities to *H. sanguineus*.

Hebling, N.J. 1982. Desenvovimento dos primeiros estagios juvenis de *Panopeus herbstii*. Milne-Edwards, 1834 (Crustacea, Decapoda, Xanthidae), criados em laboratorio. Naturalia, Sao Paulo, 7:177-188.

Describes (in Portuguese) and provides figures of juvenile stages of *P. herbsteii*

Hogarth, P.J. 1978a. Pattern polymorphism and predation in the shore crab, *Carcinus maenas*. Crustaceana 28:316-319.

Includes figures of juvenile carapace coloration patterns for *C. maenas*.

Hogarth, P.J. 1978b. Variation in the carapace pattern of juvenile *Carcinus maenas*. Marine Biology 44:337-343.

Includes photographs and descriptions of juvenile *C. maenas*.

Hwang, S.G., C. Lee, and C.H. Kim. Complete larval development of *Hemigrapsus sanguineus* (Decapoda, Brachyura, Grapsidae) reared in laboratory. The Korean Journal of Systematic Zoology 9(2):69-86.

Details through megalopal stage, however does not discuss juvenile stages except number of days until first and second stages.

Hwang, S.G. and C.H. Kim. 1995. Zoeal stages and megalopa of *Hemigrapsus penicillatus* (De Haan, 1835) (Decapoda, Brachyura, Grapsidae) reared in the laboratory. The Korean Journal of Systematic Zoology vol.11, No.3:389-408.

Describes megalopal stage of *H. penicillatus* and compares with *H. sanguineus*

Kurata, H. 1968. Larvae of Decapoda Brachyura of Arasaki, Sagami Bay – II. *Hemigrapsus sanguineus* (De Haan) (Grapsidae). Bull. Tokai. Reg. Fish. Res. Lab. No.56:161-165.

Describes larval through megalopal stages (in Japanese – only Abstract in English), with figures. HOWEVER, the figure of the megalopal stage does not match others' descriptions - in particular the frontal margin in this figure strongly protrudes, whereas in other descriptions the frontal margin is bilobed.

Johnson, W.S. and D.M. Allen. 2005. Zooplankton of the Atlantic and Gulf Coasts: A Guide to Their Identification and Ecology. Johns Hopkins University Press.

Includes line drawings and descriptions of larvae and megalopae, pointing out important features for identification. P.210-225.

Lebour, M.V. 1928. The larval stages of the Plymouth Brachyura. Proceedings of the Zoological Society of London.473-560.

Describes related crabs.

Martin, J.W., D.L. Felder, and F.M. Truesdale. A Comparative Study of Morphology and Ontogeny in Juvenile Stages of Four Western Atlantic Xanthoid Crabs (Crustacea: Decapoda: Brachyura). Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, Vol. 303, No. 1117 (Jan. 30, 1984), pp. 537-604

Provides detailed morphological descriptions of late larval through juvenile to adult stages of *P. herbsteii, P. bermudensis, P. turgidus,* and *Eurypanopeus depressus.*

Muraoka, K. 1971. On the post-larval characteristics of the two species of shore crab. Researches on crustacea 4/5:224-235.

Briefly describes megalopa and first crab stages of *H. sanguineus*, with figures

Muraoka, K. 1971. On the post-larval stage of three species of the shore crab, grapsidae. Bulletin of the Kanagawa Prefecture Museum 1(4):8-17.
Describes crabs related to *H. sanguineus - Grapsus strigosus, Pachygrapsus crassipes,* and *Pachygrapsus minutus*.

Olmsted, J.M.D. and J.P. Baumberger. 1923. Form and growth of grapsoid crabs: a comparison of the form of three species of grapsoid crabs and their growth at molting. Journal of morphology 38:279-294.

Discusses crabs related to *H. sanguineus – H. oregonensis, H. nudus,* and *Pachygrapsus crassipes*.

Reilly, P.N. and S.B. Saila. 1978. Biology and ecology of the Rock crab, *Cancer irroratus* Say, 1817, in southern New England waters (Decapoda, Brachyura). Crustaceana 34(2):121-140.

Provides information on growth. No figures.

Sastry, A.N. 1977. The larval development of the Jonah crab, *Cancer borealis* Stimpson, 1859, under laboratory conditions (Decapoda Brachyura). Crustaceana 32(3):290-303.

Describes *C. borealis* megalopa. Similar to *C. irroratus*.

Sastry, A.N. 1977. The larval development of the Rock crab, *Cancer irroratus* Say, 1817, under laboratory conditions (Decapoda Brachyura). Crustaceana 32(2):155-168.

Describes *C. irroratus* megalopa. Similar to *C. borealis*.

Shen, C. J. 1935. An investigation of the post-larval development of the shore-crab *Carcinus maenas*, with special reference to the external secondary sexual characteristics. Proceedings of the Zoological Society of London.

Extensively details morphology of juvenile stages of *C. maenas*.

Stauber, L.A. 1945. *Pinnotheres ostreum*, parasitic on the American oyster, *Ostrea* (Gryphaea) *virginica*. The Biological Bulletin 88:269-291.

Includes dorsal and ventral figures of first and second stage juvenile *P. ostreum*

Wass, M.L. 1955. The decapod crustaceans of Alligator Harbor and adjacent inshore areas of northwestern Florida. Journal of the Florida Academy of Sciences 18(3):129-176.

Discusses some brachyurans in an identification key.

Williams, A. B. 1984. Shrimps, Lobsters, and Crabs of the Atlantic Coast. Smithsonian.

Includes classification and developmental/ecological descriptions of species. Primarily identification information is given for adults only.

Williamson, H.C. 1964. Cruastacea Decapoda, Larven, Pp. *In* Nordisches Plankton, Zoologischer Teil, Drittern Band: Crustacea Neudruck A. Asher & Co., Amsterdam. VI 20-588.

Includes descriptions (in German) and figures of postlarvae including Xanthidae