

Untangling the effects of size, habitat and invertebrate biodiversity on parasite prevalence in the Caribbean spiny lobster

Charlotte E. Davies^{1,2*}, Patricia Briones-Fourzán¹ and Enrique Lozano-Álvarez¹

¹ Instituto de Ciencias del Mar y Limnología, Unidad Académica de Sistemas Arrecifales, Universidad Nacional Autónoma de México, Puerto Morelos, Quintana Roo, 77580 Mexico

² *Current address:* Department of Biosciences, College of Science, Swansea University, Singleton Park, Swansea, Wales UK, SA2 8PP

*Author for correspondence, Email: CEDavies72@gmail.com, Tel: +44 1792 295446

Online resource 1.- Modified protocol; extraction of genomic DNA with Wizard Genomic Commercial Kit (Promega®)

1. Take 250 µl sample (hemolymph in 96% ethanol), centrifuge at 13,000 g for 3 min, carefully decant the supernatant and dry the precipitate for 30 min.
2. Add 223 µl of the mix to each sample (for 24 samples: 4800 µl of solution of lysate [Nuclei Lysis solution], 480 µl of proteinase K, plus 72 µl of RNase).
3. Incubate in a dry bath for 3 hours (or better, overnight) at 55 °C. Every hour vortex until the tissue is completely lysed.
4. Add 100 µl of protein precipitating solution (Protein precipitation solution) to the sample. Vortex for 20 seconds, then submerge the tube completely in ice for 10 minutes.
5. Label sterile new 1.5 ml eppendorf tubes, and add 350 µl of isopropanol to each tube.
6. After 10 minutes, centrifuge samples for 5 minutes at 13000 rpm, the protein precipitate forms a white pellet.
7. Recover the supernatant, this contains the DNA, carefully with a pipette trying not to take the precipitate. Transfer the supernatant to the tubes with isopropanol.
8. Mix gently by inverting the tube (the DNA is observed as white filaments).
9. Centrifuge for 2 minutes at 13,000 rpm, carefully decant the supernatant (the precipitate is DNA).
10. Add 500 µl of 70% ethanol, mix gently by inverting the tube several times.
11. Centrifuge at 13,000 rpm for 2 minutes.
12. Carefully decant the ethanol and invert the tube on sterile absorbent paper until the pellet is completely dry (~ 2-3 hours or overnight).
13. Add 100 µl of rehydration buffer (TE) or distilled water free of endonucleases.
14. If you need to process the samples immediately, incubate at 65 °C for 1 hour to rehydrate, then vortex, or store the DNA at 4°C overnight, vortexing afterwards.
15. Store in the freezer at -20°C.

Online Resource 2.- Number of invertebrate species (in alphabetical order within higher taxa) and individuals by sampling zone (Reef, Lagoon, Shallow) in Bahía de la Ascensión, registered by visual census (N = 21 quadrats, 2 m × 2 m each, per zone)

Species	Reef	Lagoon	Shallow
CNIDARIA: ACTINIARIA			
<i>Actinostella flosculifera</i> (Le Sueur, 1817)	0	0	4
<i>Condylactis gigantea</i> (Weinland, 1860)	2	0	0
ANNELIDA: POLYCHAETA			
<i>Bispira melanostigma</i> (Schmarda, 1861)	11	5	0
<i>Eupolymnia crassicornis</i> (Schmarda, 1861)	26	0	69
<i>Hermodice carunculata</i> (Pallas, 1766)	3	0	0
<i>Loimia medusa</i> (Savigny, 1822)	0	0	1
Unidentified Polyonid	2	0	0
Unidentified Sabellid	0	0	1
CRUSTACEA: CIRRIPIEDIA			
Unidentified Balanoid	0	0	1
CRUSTACEA: DECAPODA			
<i>Actaea acantha</i> (H. Milne Edwards, 1834)	4	0	2
<i>Calcinus tibicen</i> (Herbst, 1791)	14	123	4
<i>Clibanarius tricolor</i> (Gibbes, 1850)	10	212	0
<i>Epialtus</i> sp.	0	47	0
<i>Mithraculus coryphe</i> (Herbst, 1801)	43	0	0
<i>Mithraculus forceps</i> A. Milne-Edwards, 1875	2	0	0
<i>Mithraculus sculptus</i> (Lamarck, 1818)	20	57	1
<i>Paguristes anomalus</i> Bouvier, 1918	0	113	0
<i>Paguristes puncticeps</i> Benedict, 1901	13	107	0
<i>Paguristes tortugae</i> Schmitt, 1933	0	15	0
<i>Pagurus annulipes</i> (Stimpson, 1860)	9	5	87
<i>Pagurus brevidactylus</i> (Stimpson, 1859)	60	341	159
<i>Pagurus</i> sp.	0	0	2
<i>Persephona</i> sp.	0	0	4
<i>Phimochirus holthuisi</i> (Provenzano, 1961)	0	28	0
<i>Pitho lherminieri</i> (Desbonne in Desbonne & Schramm, 1867)	2	0	0
<i>Pitho mirabilis</i> (Herbst, 1794)	16	30	0
<i>Teleophrys ruber</i> (Stimpson, 1871)	1	16	0
Unidentified Xanthoid 1	0	0	1
Unidentified Xanthoid 2	0	9	0
CRUSTACEA: ISOPODA			
Unidentified Cymothoid	0	0	5
CRUSTACEA: STOMATOPODA			
<i>Lysiosquilla scabricauda</i> (Lamarck, 1818)	1	3	0
<i>Lysiosquilla</i> sp.	4	0	18

<i>Neogonodactylus oerstedii</i> (Hansen, 1895)	10	33	2
ECHINODERMA: ECHINOIDEA			
<i>Echinometra lucunter</i> (Linnaeus, 1758)	1	0	0
<i>Eucidaris tribuloides</i> (Lamarck, 1816)	1	0	0
<i>Lytechinus variegatus</i> (Lamarck, 1816)	0	3	0
<i>Meoma ventricosa</i> (Lamarck, 1816)	0	4	0
ECHINODERMA: OPHIUROIDEA			
<i>Ophioderma appressa</i> (Say, 1825)	107	102	0
<i>Ophioderma rubicunda</i> Lütken, 1856	27	25	0
<i>Ophiomastix wendtii</i> (Müller & Troschel, 1842)	7	0	0
MOLLUSCA: BIVALVIA			
<i>Brachidontes modiolus</i> (Linnaeus, 1767)	0	0	734
<i>Chione cancellata</i> (Linnaeus, 1767)	0	17	255
<i>Ctenoides mitis</i> (Lamarck, 1807)	2	1	0
<i>Isognomon alatus</i> (Gmelin, 1791)	0	1	0
<i>Pinctada imbricata radiata</i> (Leach, 1814)	0	1	0
Unidentified Bivalve	1	52	0
MOLLUSCA: GASTROPODA			
<i>Cerithium litteratum</i> (Born, 1778)	63	835	3
<i>Cerithium</i> sp.	6	118	0
<i>Cittarium pica</i> (Linnaeus, 1758)	0	23	0
<i>Fasciolaria tulipa</i> (Linnaeus, 1758)	2	5	0
<i>Fissurella</i> sp.	1	0	0
<i>Lithopoma phoebium</i> (Röding, 1798)	0	166	0
<i>Lithopoma tectum</i> (Lightfoot, 1786)	15	33	0
<i>Lobatus costatus</i> (Gmelin, 1791)	0	3	0
<i>Lobatus gigas</i> (Linnaeus, 1758)	0	1	0
<i>Prunum pruinatum</i> (Hinds, 1844)	0	0	1
<i>Smaragdia viridis</i> (Linnaeus, 1758)	0	1	0
<i>Tegula fasciata</i> (Born, 1778)	59	344	0
Unidentified Gastropod 1	0	5	0
Unidentified Gastropod 2	0	16	0
Unidentified Gastropod 3	0	3	0
MOLLUSCA: POLYPLACOPHORA			
<i>Stenoplax purpurascens</i> (C. B. Adams, 1845)	16	0	0
<i>Tonicia schrammi</i> (Shuttleworth, 1856)	2	0	0
Total individuals	563	2903	1384
Total species	35	38	20

Online Resource 3.- Similarity measures in conspicuous invertebrate fauna within and between sampling zones in Bahía de la Ascensión, Mexico. Analysis of similarity percentage (SIMPER) for taxa in quadrats in three zones: Reef, Lagoon, and Shallow, and of dissimilarity percentage between pairs of zones. Av.Abund, average abundance; Av.Sim, average similarity; Sim/SD, similarity/standard deviation; Contrib%, contribution in %; Cum%, cumulative contribution in %; Av.Diss, average dissimilarity; Diss/SD, dissimilarity/standard deviation. Species are listed in decreasing order of Av.Abund within each zone and Av.Diss between zones. Cum.% does not reach 100% in order to facilitate interpretation. The full names of species appear in Online Resource 2

Zone: Reef

Average similarity: 33.19

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>O. apressa</i>	1.31	11.95	1.31	36.0	36.0
<i>M. coryphe</i>	0.74	3.60	0.60	10.8	46.9
<i>C. litteratum</i>	0.75	3.42	0.52	10.3	57.2
<i>P. brevidactylus</i>	0.76	3.26	0.52	9.8	67.0
<i>O. rubicunda</i>	0.57	2.70	0.47	8.1	75.1
<i>E. crassicornis</i>	0.57	2.24	0.46	6.8	81.9
<i>T. fasciata</i>	0.68	2.17	0.46	6.6	88.4
<i>M. sculptus</i>	0.44	1.51	0.33	4.5	93.0

Zone: Shallow

Average similarity: 42.44

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>P. brevidactylus</i>	1.40	17.35	1.26	40.9	40.9
<i>P. annulipes</i>	1.07	10.70	0.91	25.2	66.1
<i>C. cancellata</i>	1.10	6.98	0.58	16.5	82.5
<i>B. modiolus</i>	1.09	4.74	0.36	11.2	93.7

Zone: Lagoon

Average similarity: 41.40

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>C. litteratum</i>	2.11	10.84	1.79	26.2	26.2
<i>P. brevidactylus</i>	1.69	7.75	1.52	18.7	44.9
<i>T. fasciata</i>	1.51	5.82	0.96	14.1	59.0
<i>L. phoebium</i>	1.15	3.91	0.75	9.4	68.4
<i>C. tricolor</i>	1.09	3.12	0.66	7.5	75.9
<i>P. puncticeps</i>	0.87	2.25	0.58	5.4	81.4
<i>C. tibicen</i>	0.81	1.80	0.45	4.4	85.7
<i>O. appressa</i>	0.68	1.22	0.38	3.0	88.7
<i>P. anomalus</i>	0.65	1.00	0.33	2.4	91.1

Zones: Reef & Shallow

Average dissimilarity = 88.95

Species	Reef	Shallow	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
<i>O. apressa</i>	1.31	0	8.95	1.84	10.1	10.1

<i>B. modiolus</i>	0	1.09	7.37	0.70	8.3	18.4
<i>C. cancellata</i>	0	1.10	7.24	0.96	8.1	26.5
<i>P. annulipes</i>	0.13	1.07	7.21	1.27	8.1	34.6
<i>P. brevidactylus</i>	0.76	1.40	7.04	1.15	7.9	42.5
<i>C. litteratum</i>	0.75	0.06	4.97	0.93	5.6	48.1
<i>E. crassicornis</i>	0.57	0.45	4.83	0.98	5.4	53.5
<i>M. coryphe</i>	0.74	0	4.66	1.01	5.2	58.8
<i>O. rubicunda</i>	0.57	0	4.00	0.86	4.5	63.3
<i>T. fasciata</i>	0.68	0	3.87	0.84	4.4	67.6
<i>M. sculptus</i>	0.44	0.05	3.10	0.72	3.5	71.1
<i>Lysiosquilla</i> sp.	0.07	0.37	2.62	0.64	3.0	74.1
<i>C. tibicen</i>	0.27	0.07	2.17	0.48	2.4	76.5
<i>P. mirabilis</i>	0.22	0	1.72	0.41	1.9	78.4
<i>L. tectum</i>	0.27	0	1.52	0.47	1.7	80.2
<i>A. acantha</i>	0.12	0.10	1.39	0.45	1.6	81.7
<i>C. tricolor</i>	0.25	0	1.27	0.47	1.4	83.2
<i>N. oerstedii</i>	0.18	0.10	1.26	0.51	1.4	84.6
<i>S. purpurascens</i>	0.28	0	1.21	0.50	1.4	85.9
<i>P. puncticeps</i>	0.20	0	1.06	0.41	1.2	87.1
<i>A. flosculifera</i>	0	0.15	0.92	0.39	1.0	88.1
<i>H. carunculata</i>	0.15	0	0.91	0.40	1.0	89.2
<i>B. melanostigma</i>	0.15	0	0.86	0.33	1.0	90.1

Zones Reef & Lagoon

Average dissimilarity = 77.75

	Reef	Lagoon				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>C. litteratum</i>	0.75	2.11	6.68	1.40	8.6	8.6
<i>T. fasciata</i>	0.68	1.51	5.26	1.32	6.8	15.4
<i>P. brevidactylus</i>	0.76	1.69	5.13	1.45	6.6	22.0
<i>O. appressa</i>	1.31	0.68	4.63	1.44	6.0	27.9
<i>L. phoebium</i>	0	1.15	4.62	1.20	5.9	33.9
<i>C. tricolor</i>	0.25	1.09	4.32	1.14	5.6	39.4
<i>P. puncticeps</i>	0.20	0.87	3.49	1.04	4.5	43.9
<i>C. tibicen</i>	0.27	0.81	3.47	0.93	4.5	48.4
<i>M. coryphe</i>	0.74	0	3.02	1.02	3.9	52.2
<i>O. rubicunda</i>	0.57	0.18	2.73	0.92	3.5	55.7
<i>L. tectum</i>	0.27	0.41	2.50	0.73	3.2	59.0
<i>P. anomalus</i>	0	0.65	2.46	0.69	3.2	62.1
<i>M. sculptus</i>	0.44	0.30	2.44	0.82	3.1	65.3
<i>E. crassicornis</i>	0.57	0	2.32	0.86	3.0	68.2
<i>C. pica</i>	0	0.42	2.19	0.65	2.8	71.1
<i>Cerithium</i> sp.	0.08	0.51	2.11	0.59	2.7	73.8
<i>P. mirabilis</i>	0.22	0.23	1.67	0.57	2.1	75.9
Unid. Bivalve	0.05	0.39	1.58	0.58	2.0	78.0
<i>N. oerstedii</i>	0.18	0.25	1.35	0.55	1.7	79.7

<i>B. melanostigma</i>	0.15	0.16	1.31	0.51	1.7	81.4
<i>P. annulipes</i>	0.13	0.16	1.08	0.52	1.4	82.8
<i>Epialtus</i> sp.	0	0.27	1.02	0.40	1.3	84.1
<i>S. purpurascens</i>	0.28	0	0.88	0.49	1.1	85.2
Unid. Gastropod 1	0	0.16	0.80	0.32	1.0	86.3
<i>F. tulipa</i>	0.10	0.07	0.77	0.37	1.0	87.2
<i>L. scabricauda</i>	0.05	0.10	0.68	0.37	0.9	88.1
<i>P. holthuisi</i>	0	0.18	0.65	0.32	0.8	88.9
<i>H. carunculata</i>	0.15	0	0.60	0.41	0.8	89.7
<i>L. costatus</i>	0	0.10	0.57	0.32	0.7	90.4

Zones Shallow & Lagoon

Average dissimilarity = 87.39

Species	Shallow	Lagoon	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
<i>C. litteratum</i>	0.06	2.11	9.86	2.00	11.3	11.3
<i>T. fasciata</i>	0	1.51	6.66	1.47	7.6	18.9
<i>B. modiolus</i>	1.09	0	5.15	0.71	5.9	24.8
<i>C. cancellata</i>	1.10	0.10	5.12	0.98	5.9	30.7
<i>L. phoebium</i>	0	1.15	5.07	1.22	5.8	36.5
<i>P. annulipes</i>	1.07	0.16	4.82	1.35	5.5	42.0
<i>C. tricolor</i>	0	1.09	4.80	1.11	5.5	47.5
<i>P. brevidactylus</i>	1.40	1.69	4.07	1.11	4.7	52.2
<i>P. puncticeps</i>	0	0.87	3.78	1.01	4.3	56.5
<i>C. tibicen</i>	0.07	0.81	3.60	0.86	4.1	60.6
<i>O. appressa</i>	0	0.68	2.93	0.77	3.4	64.0
<i>P. anomalus</i>	0	0.65	2.68	0.70	3.1	67.0
<i>C. pica</i>	0	0.42	2.46	0.66	2.8	69.8
<i>L. tectum</i>	0	0.41	2.35	0.60	2.7	72.5
<i>Cerithium</i> sp.	0	0.51	2.14	0.55	2.5	75.0
<i>E. crassicornis</i>	0.45	0	1.99	0.54	2.3	77.3
<i>Lysiosquilla</i> sp.	0.37	0	1.74	0.61	2.0	79.2
Unid. Bivalve	0	0.39	1.63	0.55	1.9	81.1
<i>N. oerstedii</i>	0.10	0.25	1.32	0.51	1.5	82.6
<i>M. sculptus</i>	0.05	0.30	1.22	0.46	1.4	84.0
<i>Epialtus</i> sp.	0	0.27	1.12	0.40	1.3	85.3
<i>B. melanostigma</i>	0	0.16	1.00	0.39	1.1	86.4
<i>P. mirabilis</i>	0	0.23	0.91	0.40	1.1	87.5
Unid. Gastropod 2	0	0.16	0.90	0.32	1.0	88.5
<i>P. holthuisi</i>	0	0.18	0.70	0.32	0.8	89.3
<i>A. flosculifera</i>	0.15	0	0.66	0.39	0.8	90.1