

Opisthobranch Newsletter

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READER FORUM

Opisthobranchs Of Bahía Tortugas, Baja California Sur, Mexico (27° 41.6' N; 114° 53.3' W):

Preliminary Report On 1997-1998 Conabio-Sponsored Expeditions

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Twenty years ago, the southern distributional limits of many Californian opisthobranchs ended at La Jolla or San Diego. This was an artifact of collecting activities and politics, not biology. During the past 15 plus years, the opisthobranch fauna of the northwestern coast of the Baja California peninsula has become much better known (e.g., Bertsch, 1990, 1991 b, 1993, Bertsch & Gosliner, 1986, and Bertsch & Willan, 1986). The region is clearly part of the warm temperate faunal province of southern California, which ends approximately at Punta Eugenia. The tropical Panamic (=eastern Pacific) faunal province begins at Bahía Magdalena (Keen, 1971), leaving an ecotonal provincial level of overlap between these 2 areas.

Probably two of the most important, but least known areas, are the rocky intertidal and subtidal region[s] on the Punta Eugenia sub-peninsula and the nearby islands of Amalgua (=Cedros) and Natividad. To my knowledge, the rocky coastline around the point (from Malarrimo through Bahía Tortugas to Punta Abrejos) has only been studied systematically by one scientific research expedition, the mid 1980s joint expeditions of the California Academy of Sciences and the Universidad Autónoma de Baja California, Ciencias Marinas. Although several publications have resulted from that expedition (including Bertsch, 1985, Gosliner, 1994, Gosliner, Ghiselin and Bertsch, 1985, and data incorporated in Behrens, 1991), the fauna still remains basically unknown, although one can predict a mixture of southern California and Gulf of California species. As evidence of our lack of understanding of this region, almost no data exist on seasonal variation.

The importance of this region has been acknowledged by the Mexican government in naming the region a Zona Prioritaria for research, and establishing the Biosphere Reserve of Vizcaino here. With the support of a grant from *Comisión Nacional para el Conocimiento y el Uso de la Biodiversidad* (CONABIO), we were able to make

repeated studies in the intertidal region of Bahía Tortugas (December 1997, February and June 1998), and at subtidal sites on Islas Natividad and Cedros (June 1998). These expeditions yielded significant information about the biogeography and life histories of opisthobranch species in this region.

Total numbers of species, specimens, maximum, minimum and mean sizes (in mm), and numbers of egg masses are listed in Tables 1-4. A summary of numbers of specimens and egg masses observed at Bahía Tortugas is given in Table 5.

RANGE EXTENSIONS

We report significant range extensions of two tropical species, occurring for the first time on the outer coast of Baja California. *Pleurobranchus areolatum* (Mörch, 1863) has been recorded from numerous localities within the Gulf of California and south along the Pacific coasts of Mexico and Central America to the Galápagos Islands. It was originally described from the Caribbean, and has also been reported from east Africa (Bertsch & Smith, 1973). Our record of this species from Isla Cedros constitutes an intriguing northward range extension along the Pacific coast of the Baja California peninsula.

Although we have only one record of this species, other possible reports of this species both from this region and from the southern Californian Channel Islands (Mia Tegner, Dave Behrens, pers. comm.) suggest a thermally anomalous range occurrence of *P. areolatum* during periods of El Niño or other warming events (see Zinsmeister, 1974, and Zinsmeister & Emerson, 1979).

The following two species may also be thermally anomalous distribution records.

Spurilla neapolitana (Delle Chiaje, 1823) has been widely reported from a number of nearly circumtropical locations: on both sides of the tropical Atlantic (including the Mediterranean and Caribbean Seas), in the Pacific from Hawaii, and in the eastern Pacific only from the central portion of the Gulf of California. Its presence at Bahía Tortugas marks a significant northward range extension to the middle of the outer Pacific coastline of the Baja California peninsula.

Our records of a third species, *Aeolidiella indica* Bergh, 1888, represent an important intermediate record between its previously only known occurrences on the Pacific coast of the United States or inside the Gulf of California: San Diego, California (Sphon, 1971), and Bahía San Marte, Baja California Sur (Ferreira & Bertsch, 1975). This species is widely distributed throughout worldwide tropical waters (see Gosliner & Griffiths, 1981, and Baba, 1979)

Seasonality and Reproduction

We observed egg masses for 7 of the 20 species encountered at Bahía Tortugas. Four of these species were frequent enough to provide us with evidence of their seasonality. Eggs of *Berthella stellata* (Risso, 1826), *Spurilla neapolitana*, and *Aeolidiella indica* were surprisingly common in early winter (December); by

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contrast, the egg masses of *Aeolidiella chromosoma* (Cockerell & Eliot, 1905) were most common in early spring-summer (February and June). Especially surprising was the large number of egg masses encountered in December. It raises a number of questions: Is this typical for the intertidal region inside Bahía Tortugas and for nearby regions on the outer coastlines (e.g., Punta Eugenia and Malarrimo)? It is different from reproductive cycles at Bahía de los Angeles (inside the Gulf of California), and Pt. Loma, San Diego (Bertsch, pers. obs., and 1989, 1991a, 1995)

CONCLUSION

Obviously more data are needed to fully understand these apparent patterns. More collecting records from disparate sites need to be published or obtained so that we begin to understand the larger dynamics of opisthobranch biodiversity and biogeography.

ACKNOWLEDGEMENTS

We are grateful to CONABIO for the grant which allowed us to perform this research under the leadership of Carlos Sanchez; the assistance of the staff at the fishing cooperatives in Bahía Tortugas: including Manuel Higuera Serrano of La Purisima, and Francisco Robles of Cooperativa Natividad; to Francisco Javier Zacatzí Ayala and his family and crew at the Restaurant/Hotel Vera Cruz; and of course we respect and admire the great warmth and hospitality shown us by all the inhabitants of the area who asked us more than once, "What are you looking for?"

LITERATURE CITED

Baba, Kikutarō. 1979. Short account of the anatomy of a nudibranchiate mollusk, *Aeolidiella takanosimensis* Baba, 1930 from Japan. *The Veliger* 22 (1): 12-18.

Behrens, David W. 1983. Report of the 1982 outer Baja California Expedition. *Opisthobranch Newsletter* 15(4):18-19.

Behrens, David W. 1991. Pacific coast nudibranchs, second ed. Sea Challengers Press, Monterey, CA. vi + 107 pp.

Behrens, David W.; Gatewood, James 1986. New opisthobranch records for the west coast of Baja California. *Shells & Sea Life* 18(9):139-142.

Bertsch, Hans 1985. An international reconnaissance expedition: marine zoogeography of Baja California Sur. *Environment Southwest* 508: 18-23.

Bertsch, Hans 1989. Life history of the intertidal Californian nudibranch *Hopkinsia rosacea* MacFarland, 19805. *West. Soc. Malac., Ann. Rept.* 21: 19-20.

Bertsch, Hans 1990. Malacological explorations along the inner and outer coasts of Baja California. *West. Soc. Malac., Ann. Rept.* 22: 27.

Bertsch, Hans 1991 a. Seasonal, geographic, and bathymetric distribution of opisthobranchs at Bahía de los Angeles, Baja California, Mexico. *West. Soc. Malac., Ann. Rept.* 23: 18.

Bertsch, Hans 1991 b. Biodiversity of the dorid nudibranch (Mollusca: Opisthobranchia) fauna of the Pacific Coast of the three Californias: Systematic and zoogeographic comments. In: Murray Dailey and Hans Bertsch (eds.), *Proc. VIII International Symposium of Marine Biology, Ensenada, Baja California, Mexico.* pp. 107-115.

Bertsch, Hans 1993. Opisthobranchios (Mollusca) de la costa occidental de México. In: S.I. Salazar Vallejo y N.E. Gonzalez (eds.), *Biodiversidad Marina y Costera de México. Com. Nal. Biodiversidad y CIQRO, México.* pp. 253-270.

Bertsch, Hans 1995. A three-year phenological analysis of subtidal populations of opisthobranch mollusks at Bahía de los Angeles, Baja California, México. *Proc. Pacific Div. Amer. Assoc. for the Advancement of Science* 14 (1): 34.

Bertsch, Hans; Gosliner, Terrence M. 1986. Anatomy, distribution, synonymy, and systematic relationships of *Atagema alba* (O'Donoghue, 1927) (Nudibranchia: Doridacea). *The Veliger* 29 (1): 123-128.

Bertsch, Hans; Smith, Alberic A. 1973. Observations on three opisthobranchs (Mollusca: Gastropoda) of the La Paz area, Baja California, Mexico. *Southwestern Naturalist* 18 (2): 165-176.

Bertsch, Hans; Willan, Richard C. 1986. Occurrence of the nudibranch *Diaphorodoris lirulatocauda* in northwest Mexico. *The Festivus* 18 (10): 145.

Ferreira, Antonio J.; Bertsch, Hans 1975. Anatomical and distributional observations of some opisthobranchs from the Panamic faunal province. *Veliger* 17 (4): 323-330.

Gosliner, Terrence M. 1994. New records of Flabellinidae (Opisthobranchia: Aeolidacea) from the tropical Americas, with descriptions of two new species. *Proc. Calif. Acad. Sci.* 48 (9): 171-183.

Gosliner, Terrence M.; Ghiselin, Michael T.; Bertsch, Hans 1985. Opisthobranch mollusks of the Punta Eugenia region, with a discussion of their biogeographic affinities. *West. Soc. Malac., Ann. Rept.* 17: 11.

Gosliner, Terrence M.; Griffiths, Roberta J. 1981. Description and revision of some South African aeolidacean Nudibranchia (Mollusca, Gastropoda). *Ann. So. African Mus.* 84 (2): 105-150.

Keen, A. Myra 1971. Sea shells of tropical West America: marine mollusks from Baja California to Peru. Stanford Univ. Press, Stanford, CA. xiv + 1064 pp.

Sphon, Gale G. 1971. New opisthobranch records for the eastern Pacific. *The Veliger* 13 (4): 368-369.

Zinsmeister, William J. 1974. A new interpretation of the thermally anomalous molluscan assemblages of the California Pleistocene. *Journ. Paleo.* 48 (1): 84-94.

Zinsmeister, William J.; Emerson, William K. 1979. The role of passive dispersal in the distribution of hemipelagic invertebrates, with examples from the tropical Pacific Ocean. *The Veliger* 22 (1): 32-40.

Table 1. Records for December 1997, Bahía Tortugas

Species	#Specimens	Max. mm	Min. mm	Mean mm	Egg Masses
<i>Berthella stellata</i>	55	37	5	14.8	31
<i>Conualevia alba</i>	7	23	6	11.7	
<i>Sal y pimienta</i> (= <i>Discodoris</i> sp. 1, Behrens, 1991)	12	32	9	19.4	3
<i>Taringa</i> (=Species 120, Behrens, 1991)	1	26			
Red dorids	2	36	23	29.5	3
<i>Dendrodoris krebsii</i>	1	17			
<i>Cuthona</i> sp. A	1	5.5			
<i>Spurilla neapolitana</i>	6	39	24	31.2	8
<i>Aeolidiella chromosoma</i>	2	8	7	7.5	
<i>Aeolidiella indica</i>	66	18	4	8.7	39

Density, Species per hour: 2.2

Density, Specimens per hour: 34.2

Table 2. Records For February 1998, Bahía Tortugas

Species	#Specimens	Max. mm	Min. mm	Mean mm	Egg Masses
<i>Navanax inermis</i>	3	32	20	26.6	
<i>Aplysia californica</i>	1	180			
<i>Aplysia vaccaria</i>	1	200			
<i>Berthella stellata</i>	36	30	6	15.8	5
<i>Berthellina engeli</i>	2	32	20	26	
<i>Ancula lentiginosa</i>	1	14			
<i>Chromodoris norrisi</i>	1	13			
<i>Tyrinna evelinae</i>	25	14	4	9.4	
<i>Conualevia alba</i>	10	14	4	9.4	
<i>Sal y pimienta</i>	17	35	8	20	2
<i>Taringa</i>	1	28			
Red dorid	4	30	14	20.5	1
<i>Dendrodoris krebsii</i>	12	31	12	20.6	
<i>Cuthona</i> sp. B	1	4			
<i>Spurilla neapolitana</i>	1	40			
<i>Aeolidiella chromosoma</i>	26	22	4	11.8	21
<i>Aeolidiella indica</i>	35	13	4	8.5	11

Density, Species per hour: 2.3

Density, Specimens per hour: 21.6

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Table 3. Records For June 1998, Islas Cedros And Natividad

Species	No. of Specimens	Max. mm	Min. mm	Mean mm	Egg Masses
<i>Tylodina fungina</i> *	1	28			
<i>Pleurobranchus areolatum</i>	1	48			
<i>Berthella engeli</i>	1	23			
<i>Doridella steinbergae</i> *	1	8			
<i>Dendrodoris krebsii</i>	3	16	14	15.0	

* Isla Natividad (non-marked species from Isla Cedros)

Density, Species per hour: 3.3

Density, Specimens per hour: 4.7

Table 4. Records For June 1998, Bahía Tortugas

Species	No. of Specimens	Max. mm	Min. mm	Mean mm	Egg Masses
<i>Berthella stellata</i>	15	28	8	16.7	2
<i>Polycera atra</i>	1	22			
Sal y pimienta	1	15			
<i>Tritonia myrakeenae</i>	1	6			
<i>Aeolidiella chromosoma</i>	19	19	7	11.4	7
<i>Aeolidiella indica</i>	4	16	9	10.8	2

Density, Species per hour: 2.8

Density, Specimens per hour: 18.9

Table 5. Number Of Specimens And Egg Masses, Bahía Tortugas (December 1997, February And June 1998)

Species	Number of Specimens			Number of Egg Masses		
	Dec	Feb	Jun	Dec	Feb	Jun
<i>Navanax inermis</i>		3				
<i>Aplysia californica</i>		1				
<i>Aplysia vaccaria</i>		1				
<i>Berthella stellata</i>	55	36	15	31	5	2
<i>Berthellina engeli</i>		2				
<i>Ancula lentiginosa</i>		1				
<i>Polycera atra</i>			1			
<i>Chromodoris norrisi</i>		1				
<i>Tyrinna evelinae</i>		25				
<i>Conualevia alba</i>	7	10				
Sal y pimienta	12	17	1	3	2	
<i>Taringa</i>	1	1			1	
Red dorids	2	4		3	1	
<i>Dendrodoris krebsii</i>	1	12				
<i>Tritonia myrakeenae</i>			1			
<i>Cuthona</i> sp. A	1					
<i>Cuthona</i> sp. B		1				
<i>Spurilla neapolitana</i>	6	1		8		
<i>Aeolidiella chromosoma</i>	2	26	19		21	7
<i>Aeolidiella indica</i>	66	36	4	39	11	2

PERSONAL NOTES

From Erwin Köhler: Some words to my trip to the Philippines Oct.-Nov. 1998. The first question from many friends of mine is "What about coral-bleaching?" I have to say, there is almost none at the dive-sites I've seen! Very few of *Acropora* sp. and some shallow

Echinopora sp. are white, but NO ONE was covered with algae. The temperature was 32°C in October and 28°C at the end of November - I think, that's ok. Now to the places I've been: 4 day at Negros, dive Center: "El Dorado": <http://www.negros.net.ph/eldorado>

Almost black beach, only few coral blocks but many slugs. If you go there, ask for Rolf, an excellent guide and uw-photographer. The rest of the time did I spend at Panglao, near Bohol, at "Atlantis Dive Center" (website doesn't work), with trips to Balicasag, Pamilacan and Siquijor. At Panglao is a very good housereef with a drop-off 6-17m, here you find everything, and a close divesite called BBC (for Bohol Beach Club) even better than the housereef (I did most of the dives at BBC).

Here's the listing of the slugs I've seen and shot:

Ardeadoris egretta (Rudman, 1984)

Chelidonura sp. (Genus: A. Adams, 1850)

Chelidonura varians (Eliot, 1903)

Chromodoris annae (Bergh, 1877)

Chromodoris geometrica (Risbec, 1928)

Chromodoris kuniei (Pruvot-Fol, 1930)

Chromodoris magnifica (Quoy & Gaimard, 1832)

Chromodoris reticulata (Pease, 1866)

Cuthona sp. (Genus: Alder & Hancock, 1855)

Dendrodoris carbunculosa (Kelaart, 1858)

Discodoris boholiensis (Bergh, 1877)

Dolabella auricularia (Lightfoot, 1780)

Elysia ornata (Swainson, 1840)

Flabellina bicolor (Kelaart, 1858)

Flabellina exoptata (Gosliner & Willan, 1991)

Flabellina rubrolineata (O'Donoghue, 1929)

Glossodoris atromarginata (Cuvier, 1804)

Glossodoris cincta (Bergh, 1888)

Glossodoris pallida (Rüppell & Leuckart, 1831)

Glossodoris rufomarginata (Bergh, 1890)

Gymnodoris subflava (Baba, 1949)

Halgerda malessio (Carlson & Hoff, 1993)

Hexabranchnus sanguineus (Rüppell & Leuckart, 1828)

Hypselodoris bullocki (Collingwood, 1881)

Hypselodoris maculosa (Pease, 1871)

Hypselodoris purpureomaculosa (Hamatani, 1995)

Jorunna funebris (Kelaart, 1858)

Kentrodoris rubescens (Bergh, 1876)

Nembrotha chamberlaini (Gosliner & Behrens, 1997)

Nembrotha cristata (Bergh, 1877)

Nembrotha kubaryana (Bergh, 1877)

Nembrotha lineolata (Bergh, 1905)

Nembrotha milleri (Gosliner & Behrens, 1997)

Nembrotha sp. (Genus: Bergh, 1877) 1

Nembrotha sp. (Genus: Bergh, 1877) 2

Nembrotha sp. (Genus: Bergh, 1877) 3

Nembrotha sp. (Genus: Bergh, 1877) 4

Notodoris minor (Eliot, 1904)

Pectenodoris trilineata (Adams & Reeve, 1850)

Phillinopsis gardineri (Eliot, 1903)

Phyllidia coelestis (Bergh, 1905)

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Phyllidia elegans (Bergh, 1869)
Phyllidia ocellata (Cuvier, 1804)
Phyllidia varicosa (Lamarck, 1801)
Phyllidiella pustulosa (Cuvier, 1804)
Phyllidiella rudmani (Brunckhorst, 1993)
Phyllidiopsis shireenae (Brunckhorst, 1990)
Phyllidiopsis krempfi (Pruvot-Fol, 1957)
Phyllidiopsis striata (Bergh, 1889)
Placobranchus ocellatus (Van Hasselt, 1824)
Pleurobranchus forskali (Rüppell & Leuckart, 1828)
Risbecia tryoni (Garrett, 1873)
Thecacera picta (Baba, 1972) (no photo, film finished)
Thuridilla bayeri (Er. Marcus, 1965)

There was one more slug with no ID - even no idea.

The uncommon ones were identified by Bill Rudman at his Sea Slug Forum

<http://www.austmus.gov.au/science/division/invert/mal/forum/index.htm> many thanks to him.

I plan a 3 week trip to Cyprus in March, 1999.. I hope to find some invaders from the Red-Sea and some more Mediterranean sea slugs. - Medslugs.Koehler@t-online.de

From Jeff Goddard: I've got a new address: Dr. Jeffrey H.R. Goddard, Marine Science Institute; University of California; Santa Barbara, CA 93106; (805) 893-8083(w); (805) 730-9239(h); goddard@lifesci.ucsb.edu

I moved down here early last fall to be with my sweetie (we're getting married this fall) and am working as a post-doc in Armand Kuris's lab on potential biocontrol of the European green crab using a rhizocephalan barnacle parasite. I managed to get out in the tidepools a little bit this past fall and was also able to examine some fresh subtidal collections and have found a few opisthobranchs, including *Aeolidiella oliviae*, *Chromodoris macfarlandi*, *Eubranchus olivaceus*, and lots of *Aplysia californica*, *A. vaccaria*, *Haminoea*, *Navanax*, and *Flabellina iodinea*. I've been collecting some data on the developmental mode of these species and will pursue this work further as time permits. I'm off to the University of Miami in a few weeks to give a seminar on nudibranchs as predators and prey and will be back at the Oregon Institute of Marine Biology this summer to teach a short course on the coastal biology of Oregon. That's the news for now; hope all is well with you.

From Sara Black Banks: Thanks for the interest in our research! I am currently working on subspeciation in *Aplysia*. I am a student and research assistant who has been obsessed with *Aplysia* since the age of 9 (when I thought I discovered them). I also work with Dr. Sherry Painter. We are looking at pheromones. At the University of Texas Medical Branch-Galveston, three faculty members are actively involved in *Aplysia*

research. We all study *Aplysia brasiliana* primarily, but also use *A. californica* and *A. vaccaria* as comparative models. My personal work also includes *A. cervina*. You can access more info. on Drs. Sherry Painter, James Blankenship and Gregg Nagle at <http://www2.utmb.edu/mbi>. - slug98@hotmail.com

From Anthony Holley: I'm from England and have been fascinated by, hunted and photographed nids for many years, around various parts of the world.

Sipadan is a famous little island off the east coast of Sabah, north Borneo. Malaysia's only oceanic island it rises straight up from 2000 ft, yet takes less than thirty mins to walk round!! Fabulous diving and nothing else. Turtles everywhere, plus big schools of jacks and barracuda.

Last July there were noticeably fewer nids than in previous years, but I still keep on finding *Notodoris serena*. I first snapped it ten years ago in the area and couldn't identify it, so was pleased it was finally named last year.

Thanks for your interest. I am signing off while I am away, so if you reply and want any more info it will not be answered till March! - anthony.holley@virgin.net

From Sérgio Ávila: I'm working at the University of the Azores. I was wondering if there is anybody in this forum, that might have published data (preferably) about opisthobranch species occurring here in the Azores (middle Atlantic Ocean). I can provide my list of confirmed species. - avila@uac.pt

INFORMATION WANTED

From Alexandre Pimenta: I am looking for any reference on the duration of the larval phase of pyramidellid mollusks. - alexpin@hotmail.com

BIBLIOGRAPHY (with Gary McDonald)

- Alexeeva, Vera; Borovikov, Dmitry; Miller, Mark W; Rosen, Steven C; Cropper, Elizabeth C. 1998-10.** Effect of a serotonergic extrinsic modulatory neuron (MCC) on radula mechanoafferent function in *Aplysia*. *Journal of Neurophysiology* 80(4):1609-1622.
- Alkon, Daniel L; Favit, Antonella; Nelson, Thomas 1998-09.** Evolution of adaptive neural networks: The role of voltage-dependent K⁺ channels. *Otolaryngology - Head and Neck Surgery* 119(3): 204-211. [*Hermisenda crassincornis*]
- Baba, Kikutarō 1998.** Anatomical Review of *Archidoris tricolor* (Baba, 1938) (Opisthobranchia: Nudibranchia: Dorididae) with the Description of a New Species from Japan. *Venus* 57(3):155-160; figs. 1-5. [English, Japanese summary; *Archidoris sugashimae* n.sp.; *Siraius*, *Austrodoris*, *Anisodoris*, *Neodoris*]
- Barnich, Ruth; Uthe, Daniela 1998-03.** The gymnosomata (Gastropoda: Opisthobranchia) in the plankton of the French Mediterranean coast. *Vie et Milieu* 48(1): 15-24. [*Pneumoderma atlanticum*; *Pneumodermapsis canephora*; *Pneumodermapsis ciliata*; *Pneumodermapsis paucidens*]
- Boudko, D.Y.; Switzer Dunlap, M.; Hadfield, M.G. 1999-01-05.** Cellular and subcellular structure of anterior sensory pathways in *Phestilla sibogae* (Gastropoda, Nudibranchia). *Journal Of Comparative Neurology* 403(1):39-52.
- Brown, Glen D. 1998-10.** Nonassociative learning processes affecting swimming probability in the seaslug *Tritonia diomedea*: Habituation, sensitization and inhibition. *Behavioural Brain Research* 95(2): 151-165.
- Bryan, P.J; McClintock, J.B; Baker, B.J. 1998-09.** Population biology and antipredator defenses of the shallow-water Antarctic nudibranch *Tritoniella belli*. *Marine Biology* 132(2):259-265.