NOTES

A new molecular technique for identifying field collections of zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) veliger larvae applied to eastern Lake Erie, Lake Ontario, and Lake Simcoe

W. Trevor Claxton and Elizabeth G. Boulding

Abstract: The veliger larvae of two introduced species of bivalves, the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena bugensis*), are difficult or impossible to tell apart morphologically. We have developed specific dreissenid polymerase chain reaction (PCR) primers from dreissenid bivalve DNA sequences, which amplify a region of the cytochrome *c* oxidase subunit I mitochondrial gene. Non-dreissenid mtDNA, as found in field-collected veliger samples, was not amplified by these new PCR primers. The DNA sequence of this region distinguishes zebra mussel from quagga mussel larvae. Restriction digests of this region using the enzyme *Scr*FI showed no intraspecies variation in restriction pattern. We used this technique to distinguish the species of veliger larvae collected in eastern Lake Erie, Lake Ontario, and Lake Simcoe. In our limited study, no quagga mussel larvae were found in Lake Simcoe, suggesting that this mussel species has not yet spread to the Kawartha Lake system. No zebra mussel larvae were found in either Lake Erie or Lake Ontario. These preliminary results add to the growing evidence that the quagga mussel is replacing the zebra mussel in parts of the lower Great Lakes.

Résumé : Les larves véligères de deux espèces introduites de bivalves, la Moule zébrée (*Dreissena polymorpha*) et la Moule quagga (*Dreissena bugensis*) sont difficiles, voire impossibles, à distinguer par simple examen morphologique. Nous avons créé, à partir de séquences d'ADN de bivalves dreissénidés, des amorces de reactions polymerases en chaîne (RPC) spécifiques aux dreissénidés qui amplifient une région du gène mitochondrial de la sous-unité I du cytochrome *c* oxydase COI. L'ADNmt d'autres organismes trouvés dans des échantillons de larves véligères recueillies en nature n'est pas amplifié par ces nouvelles amorces RPC. La séquence d'ADN de cette région distingue la larve de la Moule zébrée de celle de la Moule quagga. Les digestions de cette région au moyen de l'enzyme de restriction ScrF1 indiquent qu'il n'y a pas de variation intraspécifique du pattern de restriction. Nous avons utilisé cette technique pour distinguer les espèces de larves véligères de l'est du lac Érié, du lac Ontario et du lac Simcoe. D'après cette étude de portée limitée, aucune larve de Moule quagga n'a été trouvée dans le lac Simcoe, ce qui indique que cette espèce n'a pas encore atteint le système hydrographique du lac Kawartha. Aucune Moule zébrée n'a été trouvée dans le lac Érié ou le lac Ontario. Ces résultats préliminaires constituent une autre preuve que la Moule quagga est en train de remplacer progressivement la Moule zébrée dans certaines parties du système inférieur des Grands Lacs.
[Traduit par la Rédaction]

Introduction

In the past decade two species of dreissenid bivalves have been introduced into the Laurentian Great Lakes. The first of these is the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), which was first found in North America in Lake St. Clair in 1988 (Hebert et al. 1989). It quickly spread throughout the Great Lakes and the St. Lawrence River (Griffiths et al. 1991). More recently it has spread to the canals and rivers of eastern North America (Martel et al. 1995). The second dreissenid mussel species was first recorded in North America from Lake Ontario (May and Marsden 1992) and tentatively named the "quagga" mussel. It was subsequently identified as *Dreissena bugensis* (Andrusov 1897) by Rosenberg and Ludyanskiy (1994) and Spidle (1994), who compared its shell morphology with that of museum specimens. The quagga mussel has so far been found in Lake Ontario, Lake Erie, and the St. Lawrence River (Mills et al. 1993). Zebra and quagga mussels show considerable overlap in distribution, and as quagga mussel

Received June 17, 1997. Accepted August 29, 1997.

W.T. Claxton and E.G. Boulding. [1] Department of Zoology, University of Guelph, ON N1G 2W1, Canada.

[1]Author to whom all correspondence should be addressed (e-mail: boulding @uoguelph.ca).

Table 1. Sample sizes, collection sites, dates, and species identification of zebra and quagga mussel veligers.

Collection site	Date	Station No.	Depth (m)	No. of zebra mussel veligers	No. of quagga mussel veligers
Long Point Bay, Lake Erie	Sept. 11, 1995	1	23	0	10
		2	23	0	10
		3	23	0	10
		4	12	0	6
		5	12	0	10
		6	12	0	10
		7	3	0	4
		8	3	0	4
		9	3	0	10
Hamilton Harbour, Lake Ontario	Oct. 11, 1995	1	Surface	0	6
Beaverton, Lake Simcoe	July 10, 1996	1	Surface	18	0

colonization continues, this overlap is likely to increase. Both zebra and quagga mussels are native to rivers in the Black Sea region (Rosenberg and Ludyanskiy 1994), and are thought to have been introduced to North America by the discharge of ballast water from transoceanic vessels (Hebert et al. 1989; May and Marsden 1992).

The zebra and quagga mussel have similar life histories, which include a planktonic veliger larva. Veliger larvae develop several hours after fertilization of the egg and remain free-swimming for a period of between 1 week (Shevtsova 1968) and 5 weeks (Walz 1975). During this period, dreissenid larvae from different locations and species may mix and be transported over large distances by currents or as the result of surface wind effects and other environmental factors (Smylie 1994). As a result, the species composition of larvae and adults may differ considerably at a given location (Smylie 1994). Clearly, identification of veliger larvae to the species level is important in understanding Great Lakes larval ecology. Discriminating zebra and quagga veliger larvae by using morphological characters is difficult if not impossible, even for experts (Nichols and Black 1994; A. Martel, unpublished data; B.S. Baldwin, unpublished data), therefore a simple, economic genetic technique for separating these species would be invaluable.

In a previous study, Claxton et al. (1997) showed that adult and postlarval zebra and quagga mussels could be identified to the species level using restriction fragment length polymorphisms (RFLPs) of the cytochrome *c* oxidase subunit I (COI) mitochondrial gene. RFLP is an extremely powerful tool for distinguishing between species lacking obvious morphological differences (Avise 1994). Restriction enzyme digestion of mtDNA results in cleavage at known restriction sites specific to each enzyme. Analysis of the restriction fragment patterns can be used to detect restriction-site polymorphisms between species. As a result, it is often possible to use these patterns to distinguish between species.

Using the *NIa*IV restriction enzyme, Baldwin et al. (1996) showed that RFLP analysis could be used to distinguish between *Dreissena polymorpha* and *Dreissena bugensis* veligers that had been cultured in the laboratory and extensively cleaned before analysis. However, in their study, COI mtDNA was amplified using universal polymerase chain reaction (PCR) primers developed by Folmer et al. (1994) that amplify mtDNA from other invertebrates, including crustaceans. In our current study, we were unable to use the technique outlined by Baldwin et al. (1996) to identify field-collected veliger larvae because of contamination by non-dreissenid mtDNA found in field-collected veliger samples. As a result, we developed a new RFLP analysis technique that utilizes mtDNA PCR primers, designed from zebra and quagga mussel COI sequences, to amplify mtDNA fragments from individual veligers. These PCR primers were designed to prevent amplification of non-dreissenid DNA. We use this technique to assess the species composition of field-collected veliger larvae from Lake Erie, Lake Ontario, and Lake Simcoe.

Materials and methods

Field sampling of dreissenids

The adult zebra and quagga mussels used in this study were collected on July 12, 1995, from Long Point Bay, Lake Erie, at a depth of 2 m. Samples were placed on ice and immediately transported to the University of Guelph for analysis.

Veliger samples were collected from Long Point Bay, Lake Erie; Hamilton Harbour, Lake Ontario; and Beaverton Marina, Lake Simcoe. Veliger samples collected from Lake Erie were collected on September 11, 1995, from nine sites and three depths in Long Point Bay (Table 1). Sites 1–3 were located approximately 30 km south of the Nanticoke Generating Station, at a depth of 23 m; sites 4–6 were located near sites 1–3, 30 km south of the Nanticoke Generating Station, at a depth of 12 m; and sites 7–9 were located approximately 500 m offshore of the Nanticoke Generating Station, at a depth of 3 m. At each site, water was collected at the appropriate depth using a 5-L Van Dorn bottle. One water sample was collected at each site. Water samples were passed through a plankton net with 53-μm mesh. Veliger samples were rinsed with distilled water into 22-mL plastic vials, then placed immediately on ice for transport to the University of Guelph.

Veliger samples were collected from one depth of Lake Ontario on October 11, 1995, from one site located in Hamilton Harbour at the Canadian Centre for Inland Waters (Table 1). Water samples were collected from the surface using a 20-L bucket. In total, three water samples were collected. Water and veliger samples were treated in the same fashion as those collected in Long Point Bay.

Veliger samples collected from Lake Simcoe were collected on July 10, 1996, from one site located at the Beaverton Marina (Table 1).

Water samples were collected from the surface using a 20-L bucket. In total, three water samples were collected. Water and veliger samples were treated in the same fashion as those collected in Long Point Bay.

Separation of veliger larvae

Samples were viewed and sorted using a stereomicroscope at 32× magnification, using cross-polarized light, as described by Johnson (1995). This method allows for easy recognition of larvae even if foreign debris is present in the samples. Single larvae were removed from the sample and placed in graduated 1.5-mL tubes using a Pasteur pipette. A small amount of sample water was also unavoidably transferred with the veliger larvae. Distilled water was added to each tube after the transfer of the veliger larvae, to bring the total volume in each tube to 100 µL. Only larvae at the "D-shaped" stage were used for this study (Hopkins and Leach 1993).

Isolation of dreissenid mtDNA

Adult mtDNA was isolated as described by Claxton et al. (1997). To extract larval mtDNA, 300 µL of a homogenization buffer solution containing 1.25× SSC (1.87 M sodium chloride and 0.019 M citric acid (trisodium dihydrate)), 1.37% sodium dodecyl sulfate (SDS), and 0.5 mg/mL proteinase K was added to each 1.5-mL tube containing a larva. Samples were then incubated at 55°C for 12 h. The homogenate was then mixed with 130 µL of 5M NaCl and 44 µL of 24:1 methylene chloride : isoamyl alcohol (MCIA), and centrifuged at $2000 \times g$ for 5 min, then at $6000 \times g$ for 10 min. The supernatant was removed and 400 µL of MCIA added. The mixture was then centrifuged at $6000 \times g$ for 5 min. Ethanol precipitation was carried out on the resulting supernatant. The dried pellet was dissolved in 13 µL of sterile distilled water.

Development of dreissenid mtDNA primers and PCR

Initially we used Folmer's universal PCR primers to amplify the COI mitochondrial gene fragment from our veliger samples (Folmer et al. 1994). Restriction digests of the PCR fragment produced a zebra or quagga mussel restriction pattern plus either a 710 base pair (bp) fragment that was resistant to digestion or an unidentified pattern superimposed over the dreissenid restriction pattern. This suggested that the field-collected veliger samples contained non-dreissenid mtDNA, which was amplified by the Folmer PCR primers.

To overcome this problem, we designed PCR primers at regions of the COI sequence that were conserved between zebra and quagga mussels, using sequence data determined by Baldwin et al. (1996). The new primers were used to amplify the COI mitochondrial gene fragment from the veliger samples previously used with the Folmer PCR primers. The new primer pair amplified a 608-bp fragment of the COI mitochondrial gene. The I.U.B. codes of the PCR primer pair (5′ to 3′) used were SCTTGTKGGMACRGGTTTTAGTG (dreissenid A) and GGATCTCCTAACCCTGTWGGATCAA (dreissenid B).

The PCR was performed as described by Innis et al. (1988). We used 1.0 µL of the isolated DNA as the PCR template. The concentration of $MgCl_2$ during the PCR was 4.0 mM. The PCR was carried out for 33 cycles at an annealing temperature of 50°C, the extension was carried out for 45 s at 72°C, and the denaturing step was carried out at 95°C.

Restriction analysis

Restriction analysis of the COI gene fragment of both adults and larvae was carried out using the *Scr*FI restriction enzyme. Restriction enzyme digests consisted of 4.0 µL of PCR product, 4.5 µL of sterile distilled water, 1.0 µL of 10× buffer, and 0.5 µL of *Scr*FI. Digests were incubated at 37.0°C for 18 h, mixed with xylene cyanole at 14%, and then loaded onto 2% agrose gels containing 0.04 mg of ethidium bromide/100 mL. Electrophoresis was carried out for 2 h at 60 mV in standard TBE buffer. Gels were visualized under UV light and photographed.

Results

As in our previous study, restriction analysis of the COI mitochondrial gene of the adult zebra and quagga mussels yielded two distinct restriction patterns. Adult zebra mussels showed three mtDNA fragments of approximately 50, 150, and 400 bp (Fig. 1, lanes 3 and 4), while adult quagga mussels showed four mtDNA fragments of approximately 50, 175, 200, and 250 bp (Fig. 1, lanes 1 and 2).

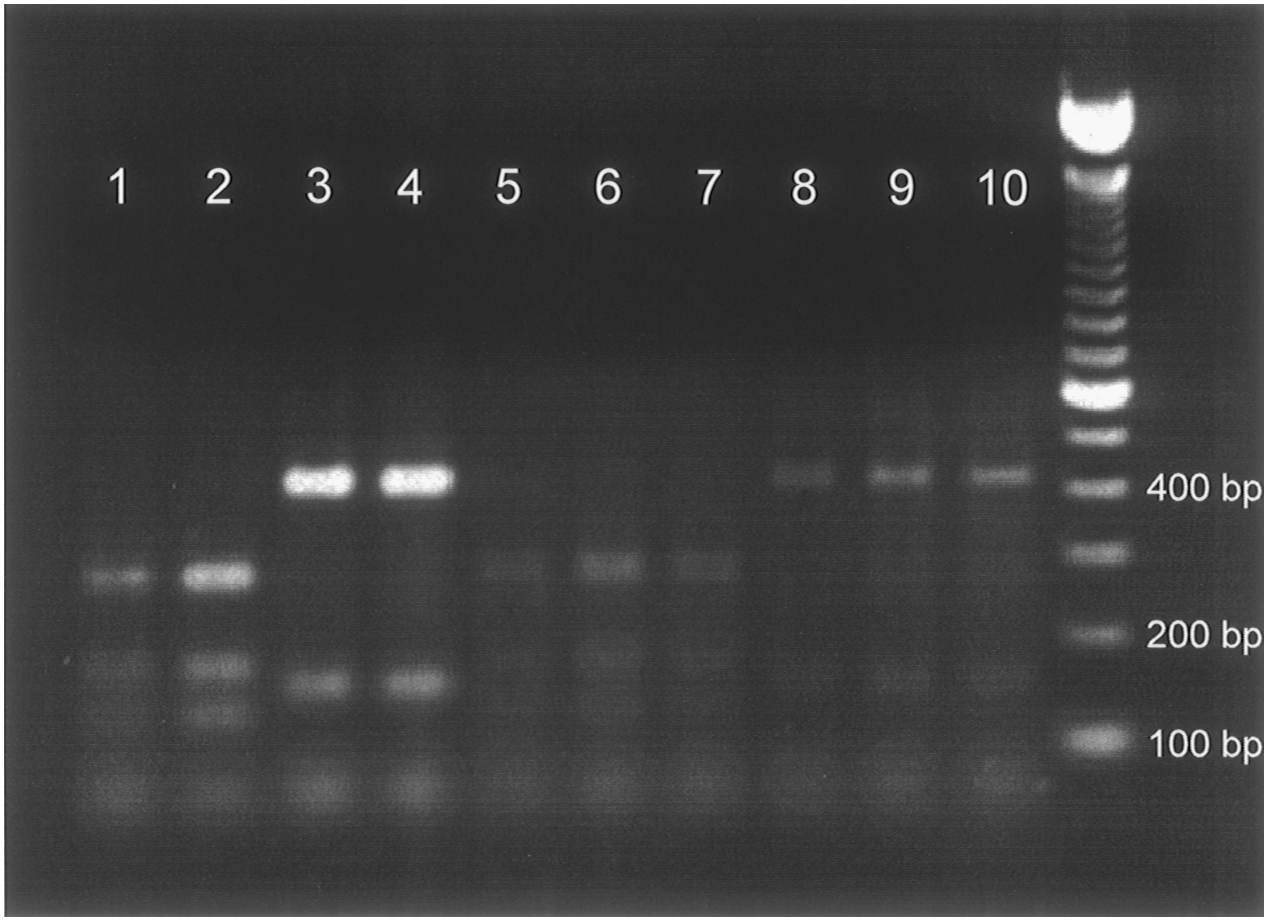
Restriction analysis of the COI mitochondrial gene of the larval dreissenids yielded the same two restriction patterns as we saw in the adults. The pattern shown in lanes 8–10 of Fig. 1 corresponds to *Dreissena polymorpha*, while the pattern shown in lanes 5–7 corresponds to *Dreissena bugensis*. RFLP analysis of veliger larvae collected at all locations yielded one of these two patterns only. No digestion-resistant fragments or alternative restriction patterns were observed for any of the veliger samples (Table 1). All larvae collected at all depths in both Lake Erie and Lake Ontario were quagga mussel veligers, while those collected from Lake Simcoe were all zebra mussel veligers.

Discussion

In this study, we developed PCR primers from two dreissenid mtDNA sequences, which were used to amplify a COI mitochondrial gene fragment of zebra and quagga mussel veligers. However, they did not amplify this region in the non-dreissenid mtDNA known to be present within our field-collected veliger samples, based on previous RFLP analysis using Folmer PCR primers. The plankton samples that contained the zebra and quagga mussel veligers also contained numerous planktonic invertebrates, therefore it is probable that the veliger DNA samples were also contaminated by these invertebrates. RFLP analysis of COI fragment from a mixture of invertebrates is extremely difficult because more than one restriction pattern may be produced. Thus, the specific primers were an improvement on the universal Folmer primers used by Baldwin et al. (1996) for veliger species identification.Owing to the lack of specificity of the Folmer PCR primers, the test veligers used by Baldwin et al. (1996) were cultured under laboratory conditions, and were extensively cleaned before DNA extraction, something which is impossible for field collections.

All of our veliger samples yielded a single restriction pattern, definitive of either zebra or quagga mussel veligers, and no intraspecies variation was noted, which simplified the RFLP analysis. Claxton et al. (1997) also noted no intraspecies variation for either adult or postlarval zebra and quagga mussels. Several studies have considered the possibilty of hybridization between zebra and quagga mussels (Nichols and Black 1993; Spidle et al. 1994, 1995). Spidle et al. (1994) showed a considerable genetic distance between species (Nei's genetic identity, $I = 0.20$), suggesting that hybridization is unlikely. In addition, Spidle et el. (1995) found no natural instances of hybridization in the lower Great Lakes, where the two species co-exist. Our present study is preliminary and was not intended to be a comprehensive investigation of dreissenid veliger species composition in the Great Lakes. However, the lack of zebra mussel veligers in the larval population of Long Point Bay and Hamilton Harbour is worth noting because a well-established population of adult zebra mussels has been

Fig. 1. Restriction digest of a fragment of the COI mitochondrial gene of zebra and quagga mussel adults and larvae. Lanes 1 and 2 represent adult quagga mussels; lanes 3 and 4 represent adult zebra mussels; lanes 5–7 represent quagga mussel veliger larvae; and lanes 8–10 represent zebra mussel veliger larvae.



documented in this area (Mills et al. 1993). Very little research has been done on the relative spawning times and fecundity of *D. polymorpha* and *D. bugensis*. However, environmental conditions such as water temperature and time of year are important factors in triggering zebra mussel spawning (Sprung 1987, 1995). Therefore, it is possible that at the time of collection, environmental conditions in Lake Erie and Ontario may have favoured quagga mussel spawning, leading to elevated numbers of their veligers in the water column.

Several authors have noted a progressive increase in the population of adult quagga mussels relative to adult zebra mussels in both Lake Erie and Lake Ontario (Dermott and Munwar 1993; Mills et al. 1993). The disproportionate number of quagga mussel veligers found in samples collected from the lakes Erie and Ontario may reflect the increasing dominance of quagga mussels in parts of these lakes.

The lack of quagga mussel veligers in Lake Simcoe is not surprising. To date, the range of quagga mussel colonization does not extend beyond Lake Ontario, Lake Erie, and the St. Lawrence River (Mills et al. 1993). Lake Simcoe has only recently been colonized by the zebra mussel (W.T. Claxton, unpublished data).

Both zebra and quagga mussels have colonized large new areas of North America over the past several years (Griffiths et al. 1991; May and Marsden 1992). Continued colonization of new lakes and rivers is likely. Therefore, identification of all life stages of both zebra and quagga mussels, even in areas currently free of dreissenids, is important for aquatic biologists and engineers. Identification of veligers is particularly important, as they pre-date adult colonization.

Acknowledgments

This research was supported by a research grant from and a collaborative project with the Natural Sciences and Engineering Research Council of Canada (NSERC) to E.G. Boulding. W. Trevor Claxton was supported by an NSERC research grant to G.L. Mackie. We also thank Dr. T. Crease for her valuable technical assistance for this study.

References

Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York and London. pp. 252–280.

Baldwin, B.S., Black, M., Sanjur, O., Gustafson, R., Lutz, R.A., and Vrijenhoek, R.C. 1996. A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves: mitochondrial COI. Mol. Mar. Biol. Biotechnol. **5**: 9–14.

Claxton, W.T., Martel, A., Dermott, R.M., and Boulding, E.G. 1997. Discrimination of field-collected juveniles of two introduced dreissenids (Dreissena polymorpha, and *Dreissena bugensis*)

using mitochondrial DNA and shell morphology. Can. J. Fish. Aquat. Sci. 1280–1288.

Dermott, R., and Munwar, M. 1993. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological impact. Can. J. Fish. Aquat. Sci. **50**: 2298–2304.

Folmer, O.M., Black, W., Hoeh, R., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. **3**: 294–299.

Griffiths, R.W., Schloesser, D.W., Leach, J.H., and Kovalak, W.P. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. **48**: 1381–1388.

Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. **46**: 1587–1591.

Hopkins, G.J., and Leach, J.H. 1993. A photographic guide to the identification of larval stages of the zebra mussel (*Dreissena polymorpha*). *In* Zebra mussels: biology, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Ann Arbour, Mich. pp. 761–772.

Innis, M.A., Myambo, K.B., Gelfand, D.H., and Brown, M.A. 1988. DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. Proc. Natl. Acad. Sci. U.S.A. **85**: 9436–9440.

Johnson, L.E. 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* spp.) veligers using cross-polarized light microscopy. Hydrobiologia, 312: 139–146.

Martel, A., Hynes, T.M., and Buckland-Nicks, J. 1995. Prodissoconch morphology, planktonic shell growth, and size at metamorphosis in *Dreissena polymorpha*. Can. J. Zool. **73**: 1835–1844.

May, B., and Marsden, J.E. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. **49**: 1501–1506.

Mills, E.L., Dermott, R.M., Roseman, E.F., Dustin, D., Mellina, E., Conn, D.B., and Spidle, A.P. 1993. Colonization, ecology, and population structure of the quagga mussel (Bivalvia: Dreissenidae) in the lower Great Lakes. Can. J. Fish. Aquat. Sci. **50**: 2305–2314.

Nichols, S.J., and Black, M.G. 1993. Veligers: zebra mussel, quagga, and hybrids. *In* Abstracts of the Third International Zebra Mussel Conference, Toronto, Ont., Canada. [Abstr.]

Nichols, S.J., and Black, M.G. 1994. Identification of larvae: the zebra mussel (*Dreissena polymorpha*), quagga mussel (*Dreissena rosteriformis bugensis*), and Asian clam (*Corbicula fluminea*). Can. J. Zool. **72**: 406–417.

Rosenberg, G., and Ludyanskiy, M.L. 1994. A nomenclatural review of *Dreissena* (Bivalvia: Dreissenidae), with identification of the quagga mussel as *Dreissena bugensis*. Can. J. Fish. Aquat. Sci. **51**: 1474–1484.

Shevtsova, L.V. 1968. Peculiarities of the reproduction and development of *Dreissena* in the Dmnepr–Krivoj–Roj canal. Zool. Zh. **4**: 70–72

Smylie, P. 1994. Growth and abundance of *Dreissena polymorpha* larvae in relation to temperature and phytoplankton abundance. M.Sc. thesis, University of Guelph, Guelph, Ont.

Spidle, A.P., Marsden, J.E., and May, B. 1994. Identification of the Great Lakes quagga mussel as *Dreissena bugensis* from the Dnieper, Ukraine, on the basis of allozyme variation. Can. J. Fish. Aquat. Sci. **51**: 1485–1489.

Spidle, A.P., Mills, E.L., and May, B. 1995. Absence of naturally occurring hybridization between the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*) in the lower Great Lakes. Can. J. Zool. **73**: 400–403.

Sprung, M. 1987. Ecological requirements of developing *Dreissena polymorpha* eggs. Arch. Hydrobiol. Suppl. **79**: 69–86.

Sprung, M. 1995. Physiological energetics of the zebra mussel *Dreissena polymorpha* in lakes. I. Growth and reproductive effort. Hydrobiologia, **304**: 117–132.

Walz, N. 1975. The settlement of larvae of *Dreissena polymorpha* Pallas on artificial substrates, Arch. Hydrobiol. Suppl. **47**: 423–431.

An intergeneric hybrid in the family Phocoenidae

Robin W. Baird, Pamela M. Willis, Tamara J. Guenther, Paul J. Wilson, and Bradley N. White

Abstract: A 60-cm female fetus recovered from a Dall's porpoise (*Phocoenoides dalli*) found dead in southern British Columbia was fathered by a harbour porpoise (*Phocoena phocoena*). This is the first report of a hybrid within the family Phocoenidae and one of the first well-documented cases of cetacean hybridization in the wild. In several morphological features, the hybrid was either intermediate between the parental species (e.g., vertebral count) or more similar to the harbour porpoise than to the Dall's porpoise (e.g., colour pattern, relative position of the flipper, dorsal fin height). The fetal colour pattern (with a clear mouth-to-flipper stripe, as is found in the harbour porpoise) is similar to that reported for a fetus recovered from a Dall's porpoise to off California. Hybrid status was confirmed through genetic analysis, with species-specific repetitive DNA sequences of both the harbour and Dall's porpoise being found in the fetus. Atypically pigmented porpoises (usually traveling with and behaving like Dall's porpoises) are regularly observed in the area around southern Vancouver Island. We suggest that these abnormally pigmented animals, as well as the previously noted fetus from California, may also represent hybridization events.

Received May 15, 1997. Accepted August 14, 1997.

R.W. Baird.[1] Biology Department, Dalhousie University, Halifax, NS B3H 4J1, Canada, and Marine Mammal Research Group, Box 6244, Victoria, BC V8P 5L5, Canada.
P.M. Willis[2] **and T.J. Guenther.** Marine Mammal Research Group, Box 6244, Victoria, BC V8P 5L5, Canada.
P.J. Wilson and B.N. White. Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.

[1] Author to whom all correspondence should be addressed (e-mail: rwbaird@is.dal.ca).
[2] Present address: Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

Résumé : Un fétus femelle de 60 cm prélevé chez un Marsouin de Dall (*Phocoenoides dalli*) trouvé mort dans le sud de la Colombie-Britannique avait comme père un Marsouin commun (*Phocoena phocoena*). Il s'agit là du premier cas d'hybridation jamais rapporté chez un phocénidé et le premier cas bien documenté d'hybridation d'un cétacé en nature. Plusieurs caractéristiques morphologiques du fétus étaient intermédiaires entre les deux espèces (par exemple le nombre de vertèbres), ou alors plus semblables aux caractéristiques du Marsouin commun qu'à celles du Marsouin de Dall (par exemple, la coloration, la position relative des nageoires antérieures, la hauteur de la nageoire dorsale). La coloration du fétus (avec une rayure nette entre la gueule et la nageoire antérieure, comme chez le Marsouin commun) était semblable à celle décrite chez un fétus recueilli chez un Marsouin de Dall au large de la Californie. Le statut hybride du fétus trouvé a été confirmé par une analyse génétique qui a révélé des séquences répétées d'ADN spécifiques des deux espèces. Des marsouins à pigmentation atypique (voyageant ordinairement avec des Marsouins de Dall et se comportant comme eux) sont observés régulièrement dans les eaux du sud de l'Île de Vancouver. Il est fort possible que ces animaux, de même que le fétus trouvé au large de la Californie, soient également des cas d'hybridation.
[Traduit par la Rédaction]

Introduction

Interspecific hybrids have been recorded in virtually every major group of vertebrates (e.g., birds, Short 1969; reptiles, Montanucci 1970; mammals, Van Gelder 1977; fish, Avise and Saunders 1984; amphibians, Spolsky and Uzzel 1984). In the order Cetacea there are a number of records of captive hybridization involving the bottlenose dolphin (*Tursiops truncatus*). Published references of captive hybrids include bottlenose dolphin crosses with the Risso's dolphin, *Grampus griseus*, false killer whale, *Pseudorca crassidens*, rough-toothed dolphin, *Steno bredanensis*, and short-finned pilot whale, *Globicephala macrorhynchus* (Antrim and Cornell 1981; Dohl et al. 1974; Nishiwaki and Tobayama 1982; Shimura et al. 1986; Sylvestre and Tasaka 1985). Bottlenose dolphin crosses with short-beaked common dolphins (*Delphinus delphis*) and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) have also been noted in captivity (D. Odell, unpublished data).

In the wild, several cases of possible or probable interspecific hybridization have also been noted. These include crosses between Risso's and bottlenose dolphins (Fraser 1940), rough-toothed and bottlenose dolphins (Simoes-Lopes et al. 1994), and narwhal (*Monodon monoceros*) and beluga (*Delphinapterus leucas*) (Heide-Jorgensen and Reeves 1993). B. Wursig (personal communication) has also observed possible hybrids between bottlenose dolphins and dusky dolphins (*Lagenorhynchus obscurus*) off New Zealand, as well as apparent interspecific matings between these two species. Of all these cases, evidence confirming interspecific hybridization has been sparse (see also Reyes 1996). In the case of blue whales (*Balaenoptera musculus*) and fin whales (*Balaenoptera physalus*), several well-documented hybrids have been reported (Arnason et al. 1991; Spilliaert et al. 1991). Documentation of pregnancies or births in hybrids, both in captivity (Odell and McClune 1997) and in the wild (Arnason et al. 1991), have demonstrated that hybrids are fertile in at least some cases.

Two members of the family Phocoenidae are found in the temperate and (or) cold waters of the North Pacific, the harbour porpoise (*Phocoena phocoena*) and Dall's porpoise (*Phocoenoides dalli)*. The ranges of these two species overlap primarily in coastal waters (cf. Barlow 1988; Jefferson 1988). While Dall's porpoises range from nearshore to open ocean, harbour porpoises are generally found in shallow (125 m or less) nearshore waters. Differences in colouration and morphology between the two species are readily apparent (Jefferson 1990; Koopman and Gaskin 1994). The behaviour of these two species around boats also differs. Dall's porpoises regularly approach vessels and ride bow waves, while harbour porpoises usually avoid vessels (Leatherwood and Reeves 1983). In the waters around southern Vancouver Island (British Columbia, Canada), both Dall's and harbour porpoises are encountered regularly (Baird 1994; Baird and Guenther 1994). Some habitat segregation appears to occur, Dall's porpoises being found in waters generally over 50 m in depth and harbour porpoises generally occurring in waters between 20 and 100 m in depth (Baird and Guenther 1994, 1995). Mixed-species groups are extremely rare, with only three sightings of mixed groups recorded in over 1500 records of the two species in the area (Marine Mammal Research Group, unpublished data).

The purpose of this note is to present details on a hybrid between these two species documented in southern British Columbia. We present evidence which suggests that hybridization between the two species may occasionally occur, and discuss the implications of such hybridization. This hybrid is the first reported interspecific cross in the family Phocoenidae, and only the second species pair in the order Cetacea in which hybridization in the wild has been well documented.

Methods

On 15 April 1994, the Stranded Whale and Dolphin Program of British Columbia (SWDP; see Baird 1994) recovered a dead 189.8-cm Dall's porpoise (Record No. SWDP94–11) from the north side of Ten Mile Point, Victoria (48º27′N, 123º15′W), near the southern tip of Vancouver Island. A postmortem examination was undertaken on 16 April 1994. A 60-cm female fetus (SWDP94–11F) was recovered from the porpoise. Measurements of the fetus were taken following the protocol of Norris (1961).

Several photographs of Dall's porpoise fetuses were obtained from the collection of the SWDP and courtesy of M. Amano (Otsuchi Marine Research Center, University of Tokyo). We compared several measurements of the fetus with those of 14 fetal Dall's porpoises (courtesy M. Amano) and 13 fetal harbour porpoises (courtesy R. Sonntag, University of Kiel, and J. Mead, Smithsonian Institution), all within the range of 40–80 cm total length.

For genetic analyses, skin samples from the mother and fetus, as well as from two additional Dall's porpoises, two harbour porpoises, and two Pacific white-sided dolphins (for controls), were used. All samples were collected from the British Columbia coast between 1991 and 1994 and stored in a solution of dimethyl sulphoxide saturated with sodium chloride. DNA extraction and restriction enzyme

Fig. 1. Photograph of the fetus (SWDP94–11F) recovered from a female Dall's porpoise collected at Victoria, B.C. Molecular-genetic techniques (see Fig. 2) demonstrated that this is a Dall's porpoise × harbour porpoise hybrid. Note the distinct mouth-to-flipper stripe, characteristic of the harbour porpoise, and the light ventral pigmentation. Photograph by R.W. Baird.

analysis were undertaken following the procedures presented in Guglich et al. (1994). Southern blot analysis using radioactively labeled genomic harbour porpoise DNA followed the procedures of Southern (1975), Westneat et al. (1988), and Feinberg and Vogelstein (1983).

Results

The fetal colour pattern was similar to that of a harbour porpoise, including a dark gray dorsal surface blending into a white ventral surface, with a clear mouth-to-flipper stripe (Fig. 1). Necropsy results showed no gross abnormalities. The total vertebral count (taken from radiographs) was 75 (±2), with 7 cervical and 15 thoracic vertebrae. It was not possible to discriminate between lumbar and caudal vertebrae because of their early stage of ossification and the difficulty of detecting the presence of chevrons in the radiographs. The tooth count (also taken from radiographs) was 25 (upper right), 26 (upper left), 24 (lower right), and 24 (lower left). Selected measurements of the fetus are presented in Table 1. Examination of photographs suggests that the relative head size of the fetus collected in our study differs from that of normal fetal Dall's porpoises. Comparisons of body proportions (Table 2) indicate that relative head sizes do differ between the two species (Mann–Whitney U test, $p < 0.001$), and the fetal porpoise from this study is most similar in size to the average found for harbour porpoises (Table 2). Dorsal fin heights, which are reported to differ between harbour and Dall's porpoises (Gaskin et al. 1974; Jefferson 1988), were also compared (Table 2). Fetal dorsal fin height ratios (dorsal fin height divided by total length) differ significantly between the two species (Mann–Whitney U test, $p = 0.01$), and the fetal porpoise from our study had a dorsal fin height ratio most similar to that of the harbour porpoise.

Repetitive DNA markers were identified in the Dall's porpoise, harbour porpoise, and Pacific white-sided dolphin samples with each of the restriction enzymes *Pst*I, *Hin*dIII, and *Msp*I. Pacific white-sided dolphin samples were included to demonstrate the difference in repetitive DNA banding patterns from those of a more distant species. Ethidium bromide stained bands produced by *Pst*I digestion were used to distinguish the harbour porpoise and Dall's porpoise (Fig. 2). These bands are from one of two types of DNA: highly repetitive DNA or mitochondrial DNA (mtDNA). Mitochondrial DNA restriction fragment length polymorphisms (RFLP) have been visualized in ethidium bromide stained DNA extracted from specific tissues of the harbour porpoise (Wang et al. 1996). The harbour porpoise specific band in the fetus can be excluded as originating from Dall's porpoise mtDNA, as it is not present in the mother and therefore the origin is paternal. The

Table 1. Selected measurements of the hybrid fetus.

	Distance (cm)	Proportion of total length
Total length (1)	60.0	1.0
Tip of upper jaw to center of eye (2)	7.8	0.13
Tip of upper jaw to end of gape (4)	4.8	0.08
Tip of upper jaw to center of blowhole (9)	6.3	0.10
Tip of upper jaw to anterior insertion of flipper (10)	14.0	0.23
Tip of upper jaw to umbilicus (12)	27.5	0.46
Tip of upper jaw to center of anus (14)	40.4	0.67
Maximum girth (22)	35.4	0.59
Anterior length of flipper (29)	9.9	0.16
Width of flipper (31)	3.9	0.06
Height of dorsal fin (32)	4.7	0.08
Width of flukes (34)	14.9	0.25

Note: Numbers in parentheses refer to Norris (1961).

Table 2. Selected comparisons of body proportions (measurement length divided by total body length) of the hybrid fetus with proportions of the parental species.

Measurement No. from Table 1 and Norris (1961)	Body proportion						
		Dall's porpoise			Harbour porpoise		
	Hybrid	Mean	SD	*n*	Mean	SD	*n*
10	0.23	0.20	0.02	14	0.24	0.02	10
32	0.08	0.10	0.01	14	0.08	0.01	13

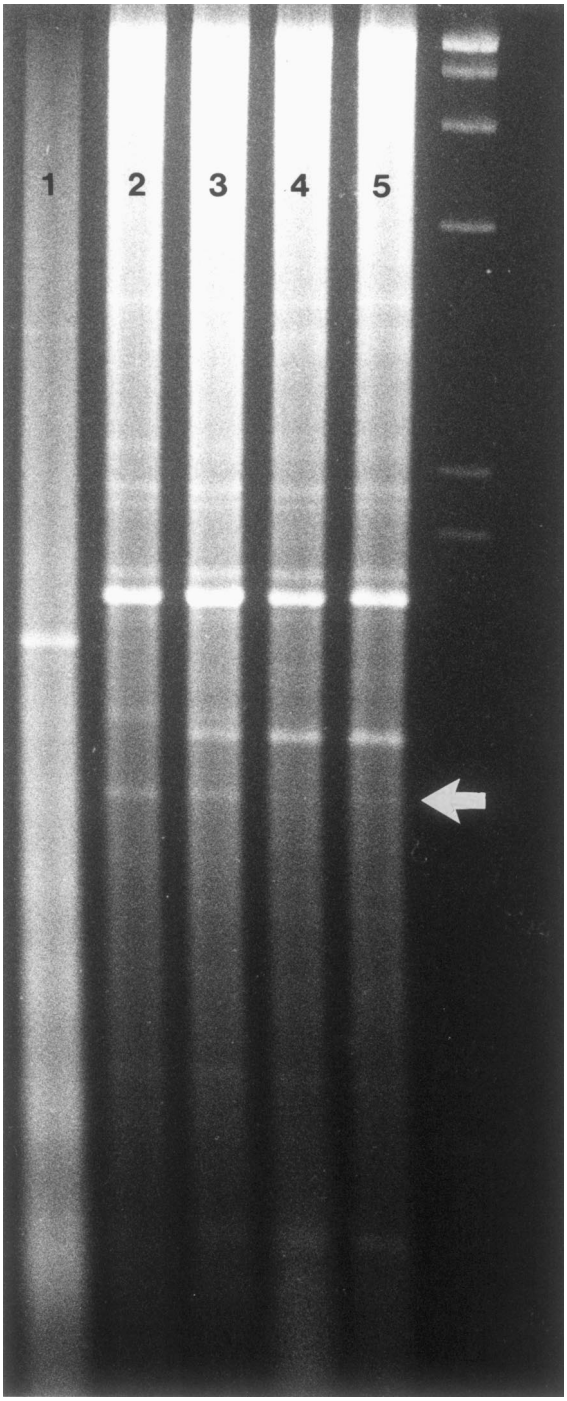
Note: Only some comparisons with parental species were made, owing to the difficulty of obtaining measurements from fetal specimens of either species. In both cases the body proportions of the hybrid were most similar to the mean value found in fetuses of the harbour porpoise, the paternal species.

banding patterns we observed are consistent with highly repetitive satellite DNA and the patterns are inconsistent with maternal inheritance of mtDNA. Southern blot analysis was also used to confirm the results. The species-specific harbour porpoise repetitive band was present in both the harbour porpoise controls and the fetus sample, but was absent in the Dall's porpoise controls (data not shown). Mitochondrial DNA RFLP patterns were not observed in the mother, the fetus, the Dall's porpoise controls, or the harbour porpoise controls.

Discussion

Species-specific DNA banding patterns demonstrated that the fetus collected from the Dall's porpoise was a hybrid between a Dall's porpoise and a harbour porpoise (Fig. 2). It was possible to make some morphological comparisons of the fetus with the parental species. The number of vertebrae in the fetus (approximately 75) appeared to be intermediate between that in the harbour porpoise (62–66; Gaskin et al. 1974) and Dall's porpoise (92–98; Jefferson 1988). Unfortunately, owing to the poor fusion of the skull, it was not possible to examine for cranial differences. Comparison of external morphometrics, in terms of relative body proportions, was difficult because of the lack of published measurements of fetuses of either species. Compilation of some unpublished fetal measurements did

Fig. 2. Ethidium bromide stained agarose gel (1.9%) of DNA (1 μg) (digested with *Pst*I) from a Pacific white-sided dolphin control (No. 1), a harbour porpoise control (No. 2), the hybrid fetus (No. 3), the Dall's porpoise mother (No. 4), and a Dall's porpoise control (No. 5). The ethidium bromide stained DNA revealed species-specific banding patterns, with a harbour porpoise specific band present in the control sample and in the fetus sample (arrow). Three other controls (one of each species) were also run and demonstrated the same banding pattern, but are not shown.



allow limited comparison of two features (Table 2), which were closer to the mean value for the harbour porpoise. Such intermediate morphological characteristics have also been noted in other cetacean hybrids (e.g., Dohl et al. 1974; Miyazaki et al. 1992; Nishiwaki and Tobayama 1982). Small sample sizes and the potential for geographic variation or allometric fetal growth (cf. Jefferson 1990; Amano and Miyazaki 1996; Read and Tolley 1997) all suggest that the current comparison should be viewed with caution, however.

The pigmentation pattern of the hybrid individual (Fig. 1) was characteristic of a normally pigmented harbour porpoise (cf. Koopman and Gaskin 1994). Harbour porpoises have a dark gray dorsal surface blending into a white ventral surface, with a clear mouth-to-flipper stripe. H.N. Koopman (personal communication) notes that harbour porpoise pigmentation patterns do not appear to change with age. The pigmentation pattern of a Dall's porpoise neonate has been described as generally similar to that of an adult, though muted, with dark gray and light gray tones later intensifying into black and white in the adult (Jefferson 1988). There is considerable variability in this typical colour pattern (e.g., Morejohn et al. 1973; Morejohn 1979), much of which relates to the amount of white on the dorsal fin and tail flukes, which varies with age and among individuals (Jefferson 1990). Unfortunately, very few descriptions or photographs of Dall's porpoise fetuses appear to exist. One 58.1-cm fetus examined from southern Vancouver Island (R.W. Baird, unpublished data) appeared to be identical with a normally pigmented Dall's porpoise neonate, though the colour pattern was even more muted. Photographs of four Dall's porpoise fetuses in the 69- to 86-cm range from Japan (provided by M. Amano) also showed a muted, though normal, pattern.

A variety of anomalously pigmented Dall's porpoises have been reported, all in areas where this species overlaps with the harbour porpoise (California, Morejohn et al. 1973; Washington, Miller 1990; British Columbia, R.W. Baird, unpublished data; Alaska, Hall 1981). These include all-gray and all-black individuals, as well as animals with pigmentation patterns similar to those of harbour porpoises. In the area around southern Vancouver Island, where our hybrid fetus was recovered, unusually pigmented (primarily gray) porpoises are frequently seen (R.W. Baird, personal observations; P.M. Willis, personal observations). Based on individually distinctive pigmentation patterns, as well as body size and location, as many as four different individuals have been seen on one day in this area (R.W. Baird, unpublished data). These individuals regularly bowride and typically surface in a similar way to Dall's porpoises. Close observation and photographs of some of these individuals (R.W. Baird, unpublished data; P.M. Willis, unpublished data) suggest that the body shape of some individuals may be intermediate between those of Dall's and harbour porpoises. All sightings of these animals have been in relatively deep water (>50 m), where Dall's porpoises are typically found, and these individuals regularly associate with normally pigmented Dall's porpoises (R.W. Baird, unpublished data; P.M. Willis, unpublished data). We suspect that these animals are hybrids between the two species, and that hybridization between the two species also occurs elsewhere in their range. A fetus collected from a Dall's porpoise off California (illustrated in Ridgway 1966 and noted as "probably term") provides some evidence to support this hypothesis, appearing virtually identical with the hybrid fetus recorded in this study. Morejohn et al. (1973) also include a drawing of a fetus with a clear mouth-to-flipper stripe, superficially similar to the hybrid fetus from our study (Fig. 1). The occurrence of hybrids between these species (considered by some to be in separate subfamilies; Barnes 1985) implies that their placement in separate genera should be reconsidered (cf. Van Gelder 1977).

The hybrid documented in this study resulted from a mating between a male harbour porpoise and a female Dall's porpoise. This directionality is consistent with predicted directionality based on differences in the potential mating strategies of the two species, as well as a decline in the size of the harbour porpoise population. Harbour porpoises exhibit reverse sexual dimorphism (females are larger than males; Gaskin et al. 1984) and males have relatively large testes (Gaskin et al. 1974), which is consistent with sperm competition and promiscuous mating (e.g., Harcourt et al. 1981; Kenagy and Trombulak 1986), while Dall's porpoises are sexually dimorphic (males are larger) and have relatively small testes (Jefferson 1990; Morejohn et al. 1973), which is consistent with unimale polygyny. Male harbour porpoises may therefore be less particular about mate choice and would be more likely to engage in interspecific matings. The harbour porpoise population in the area around southern Vancouver Island also appears to be undergoing a decline (Baird and Guenther 1994; Calambokidis and Baird 1994; Cowan 1988); such a decline in the availability of mates may also lead to directional hybridization (for example, see Grant and Grant 1997).

The role that natural hybridization might play in evolution has been both downplayed and controversial (Arnold and Hodges 1995). Yet Arnold and Hodges' (1995) review suggests that in many cases hybrids may be as fit as, or fitter than, their parents. If hybridization between harbour porpoises and Dall's porpoises is occasionally occurring in these populations, as we suspect it is, based on the presence of abnormally pigmented individuals, then the populations in southern British Columbia and Washington State may allow a detailed case study of the mechanisms and consequences of hybridization in a wild population of mammals.

Acknowledgments

We thank the individuals who assisted with the postmortem examination of the female Dall's porpoise and the fetus: R. Bates, L. Blight, S. Boehner, T. Cornish, M. McAdie, and B. Mohan. Tracy Cornish provided radiographs. Masao Amano, Jim Mead, and Ralph Sonntag provided fetal measurements for comparative purposes. Major funding for the Stranded Whale and Dolphin Program of British Columbia was provided by the World Wildlife Fund (Canada), the National Marine Mammal Laboratory (National Marine Fisheries Service, Seattle, Wash.), and the Capital Regional District (Victoria, B.C.). R.W.B was supported during the writing stage of this project by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada. Dan Odell and Sea World Inc. kindly provided information on captive-born hybrids from Sea World facilities. Mary Dillon, Nobuyuki Miyazaki, and Andy Read provided helpful comments on the manuscript.

References

Amano, M., and Miyazaki, N. 1996. Geographic variation in external morphology of Dall's porpoise, *Phocoenoides dalli*. Aquat. Mamm. 22: 167–174.

Antrim, J.E., and Cornell, L.H. 1981. *Globicephala–Tursiops* hybrid. *In* Abstracts of the Fourth Biennial Conference on the Biology of Marine Mammals, San Francisco, Calif., 14–18 December 1981. p. 4. [Abstr.]

Arnason, U., Spilliaert, R., Palsdottir, A., and Arnason, A. 1991. Molecular identification of hybrids between the two largest whale species, the blue whale (*Balaenoptera musculus*) and the fin whale (*B. physalus*). Hereditas, **115**: 183–189.

Arnold, M.L., and Hodges, S.A. 1995. Are natural hybrids fit or unfit relative to their parents? Trends Ecol. Evol. **10**: 67–71.

Avise, J.C., and Saunders, N.C. 1984. Hybridization and introgression among species of sunfish (*Lepomis*): analysis by mitochondrial DNA and allozyme markers. Genetics, **108**: 237–255.

Baird, R.W. 1994. A program to monitor the status of small cetaceans in British Columbia. Can. Wildl. Serv. Tech. Rep. Ser. No. 222. pp.122–130.

Baird, R.W., and Guenther, T.J. 1994. Status of porpoises in the British Columbia/Washington State transboundary area— a Canadian perspective. Puget Sound Notes, **34**: 5–8.

Baird, R.W., and Guenther, T.J. 1995. Account of harbour porpoise (*Phocoena phocoena*) strandings and bycatches along the coast of British Columbia. Rep. Int. Whaling Comm. Spec. Issue No. 16. pp. 159–168.

Barlow, J. 1988. Harbour porpoise, *Phocoena phocoena*, abundance estimations for California, Oregon, and Washington: I. Ship surveys. Fish. Bull. **86**: 417–432.

Barnes, L.G. 1985. Evolution, taxonomy and antitropical distributions of the porpoises (Phocoenidae, Mammalia). Mar. Mammal Sci. **1**: 149–165.

Calambokidis, J., and Baird, R.W. 1994. Status of marine mammals in the Strait of Georgia, Puget Sound and the Juan de Fuca Strait and potential human impacts. Can. Tech. Rep. Fish. Aquat. Sci. No. 1948. pp. 282–300.

Cowan, I. McT. 1988. The marine mammals of British Columbia, their status and distribution. *In* The wildlife of northern British Columbia— past, present and future. *Edited by* R.J. Fox. Spatsizi Association for Biological Research, Smithers, B.C. pp. 95–104.

Dohl, T.P., Norris, K.S., and Kang, I. 1974. A porpoise hybrid, *Tursiops* × *Steno*. J. Mammal. **55**: 217–222.

Feinberg, A.P., and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 6–13.

Fraser, F.C. 1940. Three anomalous dolphins from Blacksod Bay, Ireland. Proc. R. Ir. Acad. Sect. B Biol. Geol. Chem. Sci. **45**: 413–455.

Gaskin, D.E., Arnold, P.W., and Blair, B.A. 1974. *Phocoena phocoena*. Mamm. Species, **42**: 1–8.

Gaskin, D.E., Smith, G.J.D., Watson, A.P., Yasui, W.Y., and Yurick, D.B. 1984. Reproduction in the porpoises (Phocoenidae): implications for management. Rep. Int. Whaling Comm. Spec. Issue No. 6. pp. 135–148.

Grant, P.R., and Grant, B.R. 1997. Hybridization, sexual imprinting, and mate choice. Am. Nat. **149**: 1–28.

Guglich, E.A., Wilson, P.J., and White, B.N. 1994. Forensic identification of repetitive DNA markers to the species identification of animal tissues. J. Forensic Sci. **39**: 353–361.

Hall, J.D. 1981. Aspects of the natural history of cetaceans of Prince William Sound, Alaska. Ph.D. dissertation, University of California, Santa Cruz.

Harcourt, A.H., Harvey, P.H., Larson, S.G., and Short, R.V. 1981. Testis weight, body weight and breeding system in primates. Nature (Lond.), **293**: 55–57.

Heide-Jorgensen, M.P., and Reeves, R.R. 1993. Description of an anomalous monodontid skull from West Greenland: a possible hybrid? Mar. Mammal Sci. **9**: 258–268.

Jefferson, T.A. 1988. *Phocoenoides dalli.* Mamm. Species, **319**: 1–7.

Jefferson, T.A. 1990. Sexual dimorphism and development of external features in Dall's porpoise *Phocoenoides dalli*. Fish. Bull. **88**: 119–132.

Kenagy, G.J., and Trombulak, S.C. 1986. Size and function of mammalian testes in relation to body size. J. Mammal. **67**: 1–22.

Koopman, H.N., and Gaskin, D.E. 1994. Individual and geographical variation in pigmentation patterns of the harbour porpoise, *Phocoena phocoena* (L.). Can. J. Zool. **72**: 135–143.

Leatherwood, S., and Reeves, R.R. 1983. The Sierra Club handbook of whales and dolphins. Sierra Club Books, San Francisco.

Miller, E.J. 1990. Photo-identification techniques applied to Dall's porpoise (*Phocoenoides dalli*) in Puget Sound, Washington. Rep. Int. Whaling Comm. Spec. Issue No. 12. pp. 429–437.

Miyazaki, N., Hirosaki, Y., Kinuta, T., and Omura, H. 1992. Osteological study of a hybrid between *Tursiops truncatus* and *Grampus griseus*. Bull. Natl. Sci. Mus. Ser. A (Zool.), **18**: 79–94.

Montanucci, R.R. 1970. Analysis of hybridization between *Crotaphytus wizlizenii* and *Crotaphytus silus* (Saura: Iguanidae) in California. Copeia, 1970: 104–123.

Morejohn, G.V. 1979. The natural history of Dall's porpoise in the North Pacific Ocean. *In* Behavior of marine animals. Vol. 3. Cetaceans. *Edited by* H.E. Winn and B.L. Olla. Plenum Press, New York. pp. 45–83.

Morejohn, G.V., Loeb, V., and Baltz, D.M. 1973. Coloration and sexual dimorphism in the Dall porpoise. J. Mammal. **54**: 977–982.

Nishiwaki, M., and Tobayama, T. 1982. Morphological study on the hybrid between *Tursiops* and *Pseudorca*. Sci. Rep. Whales Res. Inst. **34**: 109–121.

Norris, K.S. 1961. Standardized methods for measuring and recording data on the smaller cetaceans. J. Mammal. **42**: 471–476.

Odell, D.K., and McClune, K.M. 1997. False killer whale, *Pseudorca crassidens* (Owen, 1846). *In* Handbook of marine mammals. Vol. 6. The second book of dolphins. *Edited by* S.H. Ridgway and R.J. Harrison. Academic Press, London. In press.

Read, A.J., and Tolley, K.A. 1997. Postnatal growth and allometry of harbour porpoises from the Bay of Fundy. Can. J. Zool. **75**: 122–130.

Reyes, J.C. 1996. A possible case of hybridism in wild dolphins. Mar. Mammal Sci. **12**: 301–307.

Ridgway, S.H. 1966. Dall porpoise, *Phocoenoides dalli* (True): observations in captivity and at sea. Nor. Hvalfangst Tid. 55: 97–110.

Shimura, E., Numachi, K., Sezaki, K., Hirosaki, Y., Watabe, S., and Hashimoto, K. 1986. Biochemical evidence of hybrid formation between the two species of dolphin *Tursiops truncatus* and *Grampus griseus*. Bull. Jpn. Soc. Sci. Fish. **52**: 725–730.

Short, L.L. 1969. Taxonomic aspects of avian hybridization. Auk, **86**: 84–105.

Simoes-Lopes, P.C., Tames, D.R., and Ximenez, A. 1994. Um novo caso de especimes intermediarios entre *Tursiops* e *Steno*? *In* Anais de 6th Reuniao de Trabalho de Especialistas em Mamiferos Aquaticos de America do Sul, Florianopolois, Brasil, 24–28 October 1994. pp. 136–137. [Abstr.]

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.

Spilliaert, R., Vikingsson, G., Arnason, U., Palsdottir, A., Sigurjonsson, J., and Arnason, A. 1991. Species hybridization between a female blue whale (*Balaenoptera musculus*) and a male fin whale (*B. physalus*): molecular and morphological documentation. J. Hered. **82**: 269–274.

Spolsky, C., and Uzzel, T. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. Proc. Natl. Acad. Sci. U.S.A. **81**: 5802–5805.

Sylvestre, J.-P., and Tasaka, S. 1985. On the intergeneric hybrids in cetaceans. Aquat. Mamm. **11**: 101–108.

Van Gelder, R.G. 1977. Mammalian hybrids and generic limits. Am. Mus. Novit. No. 2635. pp. 1–25.

Wang, J.Y., Gaskin, D.E., and White, B.N. 1996. Mitochondrial DNA analysis of harbour porpoise, *Phocoena phocoena*, subpopulations in North American waters. Can. J. Fish. Aquat. Sci. **53**: 1632–1645.

Westneat, D.W., Noon, W.A., Reeve, H.K., and Aquadro, C.F. 1988. Improved hybridization conditions for DNA "fingerprints" probed with M13. Nucleic Acids Res. **16**: 4161.