

First report of a common dolphin (*Delphinus delphis*) death following penetration of a biopsy dart

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ABSTRACT

The remote collection of skin and blubber biopsy samples from free-ranging cetaceans is a powerful technique which has been increasingly used by scientists in recent years in a wide range of applications, particularly with respect to genetic and contaminant studies. Biopsy sampling, if carried out responsibly, is known to cause low-level reactions, and is unlikely to produce long-term deleterious effects. However, this technique is not completely devoid of risk for the sampled animals, particularly for smaller odontocetes. This paper reports the death of a common dolphin in the central Mediterranean Sea, following penetration of a biopsy dart and subsequent handling. The dolphin was hit in the dorsal muscle mass below the dorsal fin by a lightweight pneumatic dart fired from a distance of 6m by a variable-power CO₂ dart projector. The methods and equipment had been previously successfully used with minimal effect on common dolphins and other species under similar conditions; it was therefore considered to be relatively uninvasive and more likely to reduce disturbance while increasing sample retrieval. However, in the reported event, a dart stuck in the dorsal muscle mass instead of recoiling as expected. Less than 2min after the hit, the dolphin began catatonic head-up sinking, and was recovered by a team member at depth. Basic medical care was given to ensure haemostasis, but the animal died 16min later. Minimal overall bleeding and a small wound in the thick muscle mass were not among the suspected causes of death. This may have been the consequence of either indirect vertebral trauma or stress. Furthermore, the dolphin had a relatively thin (7mm) blubber layer, that may have contributed to the unwanted outcome of the biopsy attempt. The author stresses that scientists should only adopt even mildly intrusive research methods after careful review and risk assessment in the light of the precautionary principle, and that their decisions must be reviewed on a regular basis according to the best available evidence.

KEYWORDS: BIOPSY SAMPLING; TECHNIQUES; COMMON DOLPHIN; MORTALITY; STRESS; MEDITERRANEAN

INTRODUCTION

The remote collection of skin and blubber biopsy samples from free-ranging cetaceans is a powerful technique which has been commonly used by scientists in recent years. It involves a minimal level of intrusiveness and analyses of the resulting samples can address many questions that previously could only be answered using samples collected from dead animals (e.g. see IWC, 1991; Aguilar and Borrell, 1994b; Lambertsen *et al.*, 1994; Barrett-Lennard *et al.*, 1996; Weller *et al.*, 1997). For example, genetic analyses of skin samples can provide information on *inter alia* social organisation, kinship, mating system, individual gender and identification, movement patterns, population size, stock identity, genetic phylogeny and variability within and among populations (e.g. Amos and Hoelzel, 1990; Palsbøll *et al.*, 1992; Baker, C.S. *et al.*, 1993; Bérubé *et al.*, 1998; Palsbøll, 1999). Analysis of the blubber portion of the samples can be used to determine contaminant levels (e.g. Aguilar and Borrell, 1994a), for various biomarker analyses and toxicological tests performed on cell cultures (e.g. Fossi *et al.*, 1992; 2000; Marsili *et al.*, 1998), and for gaining information on feeding ecology and nutritive condition through the examination of stable isotopes, fatty acids and lipid content in the blubber (e.g. Aguilar *et al.*, 1992; Kakela and Hyvarinen, 1998; Walker *et al.*, 1999; Das *et al.*, 2000).

Many authors have suggested that biopsy sampling, if carried out responsibly, is likely to cause only low-level and short-term reactions (e.g. Aguilar and Nadal, 1984 on striped dolphins, *Stenella coeruleoalba*; Cockcroft, 1994; Weller *et al.*, 1997 on bottlenose dolphins, *Tursiops truncatus*; Barrett-Lennard *et al.*, 1996 on killer whales, *Orcinus orca*; Weinrich *et al.*, 1992; Clapham and Mattila, 1993 on humpback whales, *Megaptera novaeangliae*; Jahoda *et al.*, 1996 on fin whales, *Balaenoptera physalus*; Gauthier and

Sears, 1999 on various cetacean species and see the review in IWC, 1991) and is not likely to produce any long-term deleterious effects. However, it must be remembered that biopsy sampling – as any ‘intrusive’ research approach – will entail some level of risk, however small. For example, most biopsy sampling studies involve some level of disturbance to the animals, and a variable occurrence of ‘undesired’ events. These may include missed shots, stuck darts or broken tips remaining attached to the animals, snagging of the dart retrieval line on the animal’s flukes, repeated sampling of one individual, etc. (e.g. Aguilar and Nadal, 1984; Brown, M.W. *et al.*, 1991; Weinrich *et al.*, 1992; Clapham and Mattila, 1993; Brown, M.R. *et al.*, 1994; Patenaude and White, 1995; Barrett-Lennard *et al.*, 1996; Gauthier and Sears, 1999). Most studies report a minority of ‘strong’ or ‘excited’ short-term reactions by the animals, which are generally considered to have no ‘long-term effect’ on the animals’ welfare (e.g. IWC, 1991; Aguilar and Borrell, 1994b).

The published literature does not provide accounts of remote biopsy sampling attempts having fatal consequences for any cetacean species, despite the many thousands of biopsy samples taken. More specifically, 76 common dolphin biopsies were collected with a spear gun off northwest Spain and in the Gibraltar Strait without observed fatalities (Borrell *et al.*, 1998; In press). This paper reports the death of a common dolphin (*Delphinus delphis*) following penetration of a biopsy dart and subsequent handling, during a biopsy darting attempt in the central Mediterranean Sea¹. It is hoped that making this information fully available to the wider research community will

¹ As a consequence of this event, the Tethys Research Institute has immediately interrupted its ongoing biopsy sampling activities. Subsequently, a substantial re-consideration of Tethys biopsy-related policy on the basis of the new evidence has resulted in guidelines that include abandoning biopsy darting on small cetaceans.

encourage a careful evaluation of the risks related to biopsy sampling methods, particularly as far as biopsy darting on small cetaceans is concerned.

THE COMMON DOLPHIN INCIDENT

Experience of research team

Previous experience of biopsy sampling gathered by the Tethys Research Institute over the last decade includes the remote collection of 457 samples from free-ranging cetaceans, including Mediterranean fin whales (*Balaenoptera physalus*, $n=196$), sperm whales ($n=4$), long-finned pilot whales (*Globicephala melas*, $n=1$), Risso's dolphins (*Grampus griseus*, $n=26$), bottlenose dolphins ($n=12$, including six biopsy samples taken with a CO₂ rifle, two with a hand crossbow and one with a biopsy pole), striped dolphins ($n=202$, including 88 biopsy samples taken with a biopsy pole and 114 samples of epidermal skin cells swabbed with a scratching tissue mounted on top of a biopsy pole), and common dolphins ($n=16$, including seven biopsy samples taken with a CO₂ rifle, seven with a hand crossbow and two with a biopsy pole). The biopsy tips always consisted of hollow stainless steel cylinders of various measures, according to the size of the target species. The hollow cylinder, threaded at its end, included either a hooked retention needle or a barbed dental broach. A stopper on the rear portion of the biopsy cylinder was used to control penetration to a maximum depth and cause the dart to recoil once the sample was taken. The sterilised biopsy tip was routinely disinfected prior to any biopsy attempt.

Biopsy attempts normally resulted in absent to moderate behavioural reactions elicited in the sampled animal or in the group (e.g. Jahoda *et al.*, 1996). For common dolphins, only minimal short-term reactions by the animals were recorded after a biopsy sample was obtained (Therkildsen, 2000). Typically, the biopsied animal reacted by making a hard tail

flick at the instant of dart impact (Weinrich *et al.*, 1992; Weller *et al.*, 1997), followed by a long dive. Similar short-term startle reactions were observed when the dart hit the water near the dolphin (Therkildsen, 2000), suggesting that a large component of any reaction (due to either hit or miss) is a startle response (e.g. see IWC, 1991)². The previous behavioural activity was normally resumed within minutes. The biopsied animal often re-approached the boat after being sampled, and no reactions indicative of severe stress, reduced vitality or harm were recorded. All individual common dolphins that could be photo-identified prior to a biopsy attempt were repeatedly resighted in the same area, as did the other photo-identified group members, with no indications of long-term responses or increased boat avoidance (E. Politi, pers. comm.) The same observations are true of bottlenose dolphins sampled in the same area.

The incident

In June 2000, a biopsy dart aimed at a common dolphin stuck in its muscle mass below the dorsal fin and, although not producing a lethal wound, apparently produced physical and/or physiological consequences that were fatal to the animal (Table 1). The dolphin was swimming in a group including eight other individuals³, five of which were surrounding the boat in a loose formation at the time of sampling. The event took place in the eastern Ionian Sea

² This is consistent with observations of common and striped dolphins that did not stop bowriding or shortly returned to the vessel after a sample was taken by means of a biopsy pole (Aguilar and Nadal, 1984; IWC, 1991; Tethys Research Institute, unpublished data). This minimal behavioural reaction can be due to the frenzy of the moment, as bowriding animals are in a state of arousal and may be expecting tactile stimuli (Clapham and Mattila, 1993).

³ Several members of this group were resighted in the study area during the same summer, and showed no sign of boat avoidance.

Table 1
Chronological report of the common dolphin death.

Time (min.sec.)	Notes
00'00"	Biopsy dart implant (local time 11:44hrs); the dolphin stops swimming and 'freezes', then stays at the surface with no forward movement, the body slightly arched dorsally.
01'00"	The dolphin is staying at the surface, jerks and does not swim; moderate bleeding from the wound; a crew member enters the water and stays besides the boat at about 8m from the dolphin, ready to assist; however, he does not approach to avoid further stress; after a few seconds he comes back on board.
01'15"	The dolphin shows clear signs of distress and stands in a head-up vertical position; the boat approaches at minimum speed, up to about 6m from the animal.
01'50"	The dolphin starts sinking; a crew member enters the water, dives and recovers the animal about 4-5m deep, while it was rapidly sinking in a catatonic vertical position, head up; as soon as he reaches the surface with the dolphin, the crew member extracts the dart and presses his hand on the wound to stop the bleeding; another crew member enters the water and helps to gently bring the dolphin close to the motionless boat; engine turned off; no noises on board; the rest of the dolphin group moves away.
02'13"	The dolphin is held in the water besides the inflatable and sheltered from direct sunlight; two crew members stay in the water and keep the dolphin at the surface with the blowhole out of the water; one crew member keeps pressing his hand on the wound to prevent it from bleeding; the dolphin breaths frequently, at intervals of about 10sec.
04'40"	By taking advantage of the first-aid kit on board, a small cotton 'cork' saturated with disinfectant is put on the wound as a haemostatic; a disinfected sterile gauze, kept in place by hand, is also placed on the wound; the dolphin looks quiet and stays motionless, never trying to escape or react to rescue operations; the heart – as perceived by placing a hand on the dolphin chest – beats at a slower rhythm compared to the initial time of handling; whistles and clicks are occasionally emitted; the gauze is kept on the wound to prevent bleeding.
09'30"	Two adults from the dolphin group shortly approach up to about 15m from the boat; whistles emitted by these two dolphins are heard by the crew; the wounded dolphin breaths normally and looks quiet; the heart beat is still relatively slow; the blood on the top of the wound appears to be regularly clotting.
10'48"	The dolphin makes weak movements with the tailstock for a few seconds; the crew considers releasing the animal, but decides to wait a little longer to ensure a lasting haemostasis as the wound still bleeds if the gauze is displaced.
13'00"	The heart starts beating more rapidly.
14'34"	The dolphin vomits a greyish-brown liquid; we notice that respiration may have stopped; desperate attempts to blow air into the blowhole.
15'33"	The dolphin has stopped breathing; the heart continues to beat rapidly.
16'05"	The heart stops beating.

coastal waters (38°38'09"N, 20°58'22"E), in the context of a longitudinal study on common dolphin and bottlenose dolphin behavioural ecology and ecotoxicology (Politi, 1998; Marsili *et al.*, In press). The crew comprised six people (the author, four experienced research assistants and one volunteer). The research platform was a 4.7m inflatable boat with fiberglass keel, powered by a 50HP, 4-stroke outboard engine.

The dart was fired from a variable-power dart projector (*Pneudart* Model 176B, designed for wildlife injection and marking darts) using 12g *Umarex* CO₂ capsules to pressurise a sealed chamber. The rifle had a knurled knob on the rear of the bolt as a CO₂ pressure control. The dart, stopper and dart tip were identical to those described by Barrett-Lennard *et al.* (1996) for use with killer whales. However, a smaller biopsy tip (20mm long), with a 6mm external and 5mm internal diameter was used in this case. This hollow biopsy tip typically retained a cylindrical sample of 5mm diameter⁴. The total weight of an assembled dart was 11.5g. These darts are much smaller than the darts used in other systems, and a minimum of four times lighter, to minimise the energy transferred to the target animal by biopsy strikes (Barrett-Lennard *et al.*, 1996). Our research team believed that for use with bottlenose and common dolphins, lightweight pneumatic darts shot by a variable power rifle may be more appropriate than the crossbows and spear guns used for other studies⁵. The CO₂ dart projector was also selected due to its precision. The use of large crossbows was not considered due to possible excessive impact or penetration. Poles with biopsy tips were successfully used from sailing vessels, but were ineffective from the inflatable, as the animals rarely surfaced while bowriding (which they also infrequently did), and they always showed moderate avoidance reactions when a pole protruded from our small boat.

A series of factors may have occurred to prevent the arrow from recoiling as it was expected to do. The dolphin was a subadult female (162cm, rostrum to caudal fork) that was surfacing at about 6m from the boat at the time of shooting. Although the charge regulator device of the CO₂ rifle was set to minimum power, the force provided by the first shot of a new cartridge and the perpendicular angle at which the dart penetrated the skin (approximately 90° to the dolphin's skin surface) may have increased penetration⁶. The stopper (a flat nylon nose piece to limit the depth of penetration; Barrett-Lennard *et al.*, 1996) was not large and effective enough to cause the arrow to recoil once the biopsy tip had entered the blubber. As skin and blubber biopsy samples were previously obtained from common dolphins with darts fired at a similar distance, and under largely similar conditions, it is unclear what caused the dart to enter the dorsal muscle mass. We suggest that the concomitance of several variables, rather than a single factor, may be the reason.

The dolphin looked healthy and behaved normally prior to the biopsy attempt, providing no visual evidence of it being

a sick or emaciated individual. The stomach contained nine partly-digested specimens of adult-size *Sardina pilchardus* and one part of a cephalopod beak⁷. However, an important factor to note was that the blubber layer was only 7mm thick (as compared to 18mm of a stillborn common dolphin calf found in the same area). Although the information on the blubber thickness of other freshly-stranded adult and subadult common dolphins in the area is unavailable, the blubber thickness of 20 similarly sized striped dolphins sampled in Spain ranged from 8-25mm in stranded animals (which included some diseased and emaciated animals) and 12-23mm in incidental captures (A. Aguilar, pers. comm.) Blubber thickness of striped dolphins which died during the Mediterranean morbillivirus epizootic - noteworthy because of their extreme slimmness and advanced degree of emaciation (Aguilar *et al.*, 1991; Aguilar and Raga, 1993) - was 6-15mm (A. Aguilar, pers. comm.) More information is needed to ascertain whether the thin blubber layer of the dead common dolphin was indicative of poor nutritional conditions or health problems.

DISCUSSION

It seems clear that the death of the common dolphin reported here was not a direct consequence of the wound. As confirmed by the necropsy, the biopsy dart entered the body a maximum of 40-50mm beyond the stopper, producing a wound that was a maximum of 13mm wide (i.e., the diameter of the stopper) and a minimum of 6mm wide (i.e., the diameter of the biopsy tip). The dart was stuck in the muscle mass below the base of the dorsal fin, on the upper-left side of the body, where the muscle mass was thicker. Post-mortem scrutiny of the wound revealed muscle masses surrounding the cut on all sides. Although the lack of radiological equipment made it impossible to ascertain possible fractures of the spine under these field conditions, the mechanics of the accident are not incompatible with indirect vertebral trauma due to sudden displacement of the external blubber and muscular layers. Therefore, the hypothesis of a vertebral trauma leading to subdural haemorrhage, compression of the spinal cord and subsequent paralysis of the tail muscles cannot be ruled out. These events, in turn, may have been the cause of the observed head-up sinking of the animal, the drowning of which was prevented by the prompt rescue intervention by the research team (Table 1). Blood loss appeared minimal. Death followed about 15 minutes after rescue, possibly due to vagal shock with ceased breathing and heart failure as a consequence. This severe shock may have been caused by the stuck dart, by protracted handling, or by both. Handling was initially avoided, but it was considered appropriate to try to save the animal when it suddenly started sinking in a head-up vertical position (Table 1). The possibility of a partial or progressive paralysis of tail muscles was not contemplated at the time of rescuing, as the main concern was to prevent blood loss and sinking, while trying to minimise the shock. The intention was to release the animal immediately after wound haemostasis could be granted. However, death came suddenly and unexpectedly before this could be done.

⁷ A necropsy was performed in order to gain understanding of the mechanisms that caused the death. Nematodes in the liver were found. A number of tissue samples for toxicological, histological and genetic analyses were collected and stored in liquid nitrogen, alcohol, formalin, or frozen at -20°C. Laboratory analyses are underway.

⁴ In previous experiences with both common and bottlenose dolphins, this biopsy tip never retained samples including muscle fragments or blood traces.

⁵ For instance, 'strong reactions' by small Delphinidae were reported when crossbows were used, as compared to 'mid-reactions' to spear gun biopsy sampling (IWC, 1991).

⁶ According to the dealer 'This rifle is a CO₂ operated dart projector recommended for close range shooting. It is ideal for penned animals (...) by using the power control valve, caged animals can be shot as close as two feet without injury' (Pneudart Inc., Williamsport, PA; www.pneudart.com/html/projectors.html).

Dolphin catatonia and death as a consequence of stressful events has been documented in several circumstances. These include: (1) catatonia and sinking to the bottom resulting in death by long-snouted spinner dolphins (*Stenella longirostris*) enclosed by a tuna purse-seine net (Myrick, 1988; Norris, 1991); and (2) death during handling operations aimed at live captures for the captive industry or intrusive research purposes (e.g. Klinowska and Brown, 1986; Hoyt, 1992). Individual variability may be an issue, as different individuals from the same species may react differently to similar stressors. Possibly due to individual physiological and psychological factors (Barrett-Lennard *et al.*, 1996), age, size (Peters, 1983; Gauthier and Sears, 1999), gender (Brown, M.R. *et al.*, 1994; Gauthier and Sears, 1999), reproductive state and/or hormonal conditions, illness or concurrent pathologies, behaviour at the time of sampling (IWC, 1991; Barrett-Lennard *et al.*, 1996), previous experience (Barrett-Lennard *et al.*, 1996), or other factors, some animals may be particularly fragile and can either hyper-react or 'shut off' when exposed to potentially stressful situations.

In other research arenas, animal deaths are often associated with commonly accepted research techniques. For example, immobilisation of pinnipeds and other marine and terrestrial mammals, both with and without anaesthetics, has been reported to result in the death of 3-20% of the animals handled (Loughlin and Spraker, 1989; Baker, J.R. *et al.*, 1990; Work *et al.*, 1993; Heath *et al.*, 1996). Even if conducted by experienced personnel, intrusive research at sea focusing on poorly-known species or populations implies levels of risk that may be hard to assess. There was certainly no precedent of cetacean deaths resulting from biopsy sampling in the published literature. For instance, bottlenose dolphins have been sampled with 45-60kg pull crossbows without reported accidents (Cockcroft, 1994; Weller *et al.*, 1997). 'Mid-reactions' and no fatalities have been reported for hundreds of small-sized Delphinidae (including *D. delphis* and *S. coeruleoalba*) sampled with a spear gun (IWC, 1991; A. Aguilar, pers. comm.)

Little evidence was available on the risks associated with the use of dart projectors for biopsy sampling of small-sized species or individuals prior to the event reported here. Aguilar and Nadal (1984) described the excited reaction of one striped dolphin to a stuck dart shot by a spear gun, but reported that 'the new dart proved its efficiency in about 80% of the hits in striped dolphins, and produced neither significant alterations of swimming pattern nor escape behaviour, from which it is assumed that the biopsy technique is essentially painless'. A perpendicular angle has been reported to optimise sample retrieval, minimise behavioural reactions by the target animal, and reduce the risk of stuck darts (Brown, M.W. *et al.*, 1991; Barrett-Lennard *et al.*, 1996). Patenaude and White (1995) suggested that darting should be done opportunistically at close distance to increase precision and avoid the need to adjust aim for flight curve. In addition, it has been stressed that an increased stop-collar size increases aerodynamic drag and wind resistance, and may alter the flight pattern of the arrow (Palsbøll *et al.*, 1991; Patenaude and White, 1995). As reported by Barrett-Lennard *et al.* (1996) for the darts used here, 'the small surface area of the darts was intended to limit the influence of cross winds and air friction on the flight of the dart, so that predictable trajectories could be achieved at low firing velocities'. In a study by Patenaude and White (1995) on white whale (*Delphinapterus leucas*) carcasses, stop-collar diameter was not significantly correlated to wound type. Although appropriate equipment and

techniques can clearly reduce the risks associated to biopsy sampling, it is equally clear that researchers embarking on new biopsy sampling studies should be cautious i.e. they should not over rely on experience with other species/populations and should constantly review procedures and equipment in the light of experience.

CONCLUSION

It is important to consider the present case in context. Whilst avoiding even mildly intrusive research techniques may prevent individual accidents, it would also delay or prevent the understanding of threats that may have serious consequences for entire cetacean populations. Non-destructive biopsy sampling often represents the most straightforward, effective and ethically acceptable way to evaluate threats and try to counteract the disappearance of cetacean species (thus replacing any perceived need to conduct lethal research), as is the case for common dolphin populations in the central Mediterranean Sea. That being said, scientists have an obligation to only adopt intrusive research methods after careful review and risk assessment in the light of the precautionary principle; and their decision must be reviewed on a regular basis according to the best available evidence.

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REFERENCES

- Aguilar, A. and Borrell, A. 1994a. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990-1992 Mediterranean epizootic. *Sci. Total Environ.* 154(2-3):237-47.
- Aguilar, A. and Borrell, A. 1994b. Assessment of organochlorine pollutants in cetaceans by means of skin and hypodermic biopsies. pp. 245-67. In: M.C. Fossi and C. Leonzio (eds.) *Non-Destructive Biomarkers in Vertebrates*. Lewis Publishers, Boca Raton, Florida. 457pp.
- Aguilar, A. and Nadal, J. 1984. Obtención de biopsias hipodérmicas de cetáceos en libertad. *Invest. Pesq.* 48(1):23-9. [In Spanish].
- Aguilar, A. and Raga, J.A. 1993. The striped dolphin epizootic in the Mediterranean Sea. *Ambio* 22(8):524-8.
- Aguilar, A., Borrell, A., Calzada, N. and Grau, E. 1991. The nutritive condition of striped dolphins (*Stenella coeruleoalba*) affected by the western Mediterranean epizootic. *Eur. Res. Cetaceans* [Abstracts] 5:84-6.
- Aguilar, A., Borrell, A., Calzada, N. and Grau, E. 1992. Body fat reserves in striped dolphins examined during the Mediterranean die-offs. pp.47-52. In: X. Pastor and M. Simmonds (eds.) *Proceedings of the Mediterranean Striped Dolphin Mortality International Workshop*. Palma de Mallorca, 4-5 November 1991. Greenpeace International Mediterranean Sea Project, Madrid, Spain. 191pp.

- Amos, W. and Hoelzel, A.R. 1990. DNA fingerprinting cetacean biopsy samples for individual identification. *Rep. int. Whal. Commn* (special issue) 12:79-85.
- Baker, C.S., Perry, A., Bannister, J.L., Weinrich, M.T., Abernethy, R.B., Calambokidis, J., Lien, J., Lambertsen, R.H., Urbán, J., Vasquez, O., Clapham, P.J., Alling, A., O'Brien, S.J. and Palumbi, S.R. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Natl Acad. Sci. USA* 90:8239-43.
- Baker, J.R., Fedak, M.A., Anderson, S.S., Arnbohm, T. and Baker, R. 1990. Use of a tiletamine-zolazepam mixture to immobilise wild grey seals and southern elephant seals. *Vet. Rec.* 126:75-7.
- Barrett-Lennard, L.G., Smith, T.G. and Ellis, G.M. 1996. A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. *Mar. Mammal Sci.* 12(1):14-27.
- Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo di Sciarra, G., Sears, R., Sigurjónsson, J., Urban-R, J. and Palsbøll, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. *Mol. Ecol.* 7:585-99.
- Borrell, A., Cantos, G., Pastor, T. and Aguilar, A. 1998. Organochlorine compound levels in common dolphins from the Atlantic and Mediterranean waters off Spain. *Eur. Res. Cetaceans* [Abstracts] 12:328-31.
- Borrell, A., Cantos, G., Pastor, T. and Aguilar, A. In press. Pollution by organochlorine compounds in common dolphins (*Delphinus delphis*) from the Atlantic and Mediterranean waters off Spain. *Environ. Pollut.*
- Brown, M.R., Corkeron, P.J., Hale, P.T., Schultz, K.W. and Bryden, M.M. 1994. Behavioural responses of east Australian humpback whales *Megaptera novaeangliae* to biopsy sampling. *Mar. Mammal Sci.* 10(4):391-400.
- Brown, M.W., Kraus, S.D. and Gaskin, D.E. 1991. Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. *Rep. int. Whal. Commn* (special issue) 13:81-9.
- Clapham, P.J. and Mattila, D.K. 1993. Reactions of humpback whales to skin biopsy sampling on a West Indies breeding ground. *Mar. Mammal Sci.* 9(4):382-91.
- Cockcroft, V.G. 1994. Biopsy sampling from free-range bottlenose dolphins. pp. 32-3. In: G. Notarbartolo di Sciarra, P.G.H. Evans and E. Politi (eds.) ECS Newsletter. 23-Special Issue. Proceedings of the workshop 'Methods for the Study of Bottlenose Dolphins in the Wild'. 8th Annual Meeting of the European Cetaceans Society, Maison de l'Environnement, Montpellier, 3 March 1994.
- Das, K., Lepoint, G., Loizeau, V., Debacker, V., Dauby, P. and Bouqueneau, J.M. 2000. Tuna and dolphin associations in the Northeast Atlantic: Evidence of different ecological niches from stable isotope and heavy metal measurements. *Mar. Poll. Bull.* 40(2):102-9.
- Fossi, M.C., Marsili, L., Leonzio, C., Notarbartolo-di-Sciarra, G., Zanardelli, M. and Focardi, S. 1992. The use of non-destructive biomarkers in Mediterranean cetaceans - preliminary data on MFO activity in skin biopsy. *Mar. Poll. Bull.* 24(9):459-61.
- Fossi, M.C., Marsili, L., Neri, G., Casini, S., Bearzi, G., Politi, E., Zanardelli, M. and Panigada, S. 2000. Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants. *Mar. Environ. Res.* 50:517-21.
- Gauthier, J. and Sears, R. 1999. Behavioral response of four species of balaenopterid whales to biopsy sampling. *Mar. Mammal Sci.* 15(1):85-101.
- Heath, R.B., Calkins, D., McAllister, D., Taylor, W. and Spraker, T. 1996. Telazol and isoflurane field anesthesia in free-ranging Steller's sea lions (*Eumetopias jubatus*). *J. Zoo Wildl. Med.* 27(1):35-43.
- Hoyt, E. 1992. *The Performing Orca - Why the Show Must Stop*. 1st Edn. Whale and Dolphin Conservation Society, Bath, Avon, England. 102pp.
- International Whaling Commission. 1991. Report of the *ad-hoc* working group on the effect of biopsy sampling on individual cetaceans. *Rep. int. Whal. Commn* (special issue) 13:23-7.
- Jahoda, M., Airoldi, S., Azzellino, A., Biassoni, N., Borsani, J.F., Cianfanelli, L., Lauriano, G., Notarbartolo di Sciarra, G., Panigada, S., Vallini, C. and Zanardelli, M. 1996. Behavioural reaction to biopsy-darting on Mediterranean fin whale. *Eur. Res. Cetaceans* [Abstracts] 10:43-7.
- Kakela, R. and Hyvarinen, H. 1998. Composition of polyunsaturated fatty acids in the liver of freshwater and marine ringed seals (*Phoca hispida* spp.) differs largely due to the diet of seals. *Comp. Biochem. Physiol.* 120B(2):231-7.
- Klinowska, M. and Brown, S. 1986. *A Review of Dolphinaria*. Department of Environment, London. 246pp.
- Lambertsen, R.H., Baker, C.S., Weinrich, M. and Modi, W.S. 1994. An improved whale biopsy system designed for multidisciplinary research. pp. 219-44. In: M.C. Fossi and C. Leonzio (eds.) *Nondestructive Biomarkers in Vertebrates*. Lewis Publishers, Boca Raton, Florida. 345pp.
- Loughlin, T.R. and Spraker, T. 1989. Use of Telazol (R) to immobilize female northern sea lions (*Eumetopias jubatus*) in Alaska. *J. Wildl. Dis.* 25(3):353-8.
- Marsili, L., Fossi, M.C., Notarbartolo di Sciarra, G., Zanardelli, M., Nani, B., Panigada, S. and Focardi, S. 1998. Relationship between organochlorine contaminants and mixed function oxidase activity in skin biopsy specimens of Mediterranean fin whales (*Balaenoptera physalus*). *Chemosphere* 37(8):1,501-10.
- Marsili, L., Fossi, M.C., Neri, G., Airoldi, S., Bearzi, G. and Panigada, S. In press. Skin biopsies for cell cultures from Mediterranean free-ranging cetaceans. *Eur. Res. Cetaceans* [Abstracts] 14.
- Myrick, A.C. 1988. Is tissue resorption and replacement in permanent teeth of mammals caused by stress-induced hypocalcemia? pp. 379-89. In: Z. Davidovitch (ed.) *The Biological Mechanisms of Tooth Eruption and Root Resorption*. EBSCO Media, Birmingham, Alabama.
- Norris, K.S. 1991. *Dolphin Days*. W.W. Norton and Co., New York, New York. 335pp.
- Palsbøll, P.J. 1999. Genetic tagging: Contemporary molecular ecology. *Biol. J. Linn. Soc.* 68:3-22.
- Palsbøll, P.J., Larsen, F. and Hansen, E.S. 1991. Sampling of skin biopsies from free-ranging large cetaceans in West Greenland: development of new biopsy tips and bolt designs. *Rep. int. Whal. Commn* (special issue) 13:71-9.
- Palsbøll, P.J., Vader, A., Bakke, I. and El-Gewely, M.R. 1992. Determination of gender in cetaceans by the polymerase chain reaction. *Can. J. Zool.* 70:2166-70.
- Patenaude, N.J. and White, B.N. 1995. Skin biopsy sampling of beluga whale carcasses: assessment of biopsy darting factors for minimal wounding and effective sample retrieval. *Mar. Mammal Sci.* 11(2):163-71.
- Peters, R.H. 1983. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge, UK. 329pp.
- Politi, E. 1998. Un progetto per i delfini del Mediterraneo. *Le Scienze* 360:64-9. [In Italian].
- Therkildsen, N. 2000. Immediate behavioural reactions of common dolphins (*Delphinus delphis*) and bottlenose dolphins (*Tursiops truncatus*) to remote biopsy sampling in the eastern Ionian Sea. Extended essay (Biology). International Baccalaureate School, Norway, (unpublished). 17pp.
- Walker, J.L., Potter, C.W. and Macko, S.A. 1999. The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. *Mar. Mammal Sci.* 15(2):335-50.
- Weinrich, M.T., Lambertsen, R.H., Belt, C.R., Schilling, M.R., Iken, H.J. and Syrjal, S.E. 1992. Behavioral response of humpback whales *Megaptera novaeangliae* to biopsy procedures. *Fish. Bull.* 90(3):588-98.
- Weller, D., Cockcroft, V., Würsig, B., Lynn, S. and Fertl, D. 1997. Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. *Aquat. Mamm.* 23:49-58.
- Work, T.M., DeLong, R.L., Spraker, T.R. and Melin, S.R. 1993. Halothane anaesthesia as a method of immobilizing free-ranging California sea lions (*Zalophus californianus*). *J. Zoo Wildl. Med.* 24(4):482-7.