The following supplement accompanies the article

In situ orientation of fish larvae can vary among regions

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Diver Following Methodology

The observation of behaviour of fish larvae *in situ* by divers was developed in the mid-1990s (Leis et al. 1996), and has led to a new understanding and appreciation of the behavioural capabilities of marine fish larvae in terms of swimming ability (Leis & Carson-Ewart 1997), vertical distribution (Leis 2004), orientation (Leis & Carson-Ewart 2003), the ontogeny of these behaviours (Leis 2010) and of settlement behaviour (Leis & Carson-Ewart 2002), plus 'unplanned observations' of feeding, reaction to predators and other behaviours (Leis 2006). The method was first developed for settlement-stage larvae of tropical reef fishes, but now has been applied to larvae as small as 5 mm (Leis 2010), and to temperate species both in estuaries and in the ocean (e.g., Trnski 2002, Hindell et al. 2003, Leis et al. 2006), as well as tropical species that do not live on reefs as adults (Leis et al. 2009). The method has been used with wild larvae captured by light traps or nets, and with reared larvae released into the ocean.

The method of two relatively huge divers following a small larval fish in open water has naturally been questioned in regard to the effect that the divers might have on the behaviour of the larva. Not all species are amenable to this methodology, and it has limitations in terms of duration of observation, time of day and depth, mostly due to limitations of what SCUBA divers can do. But, there are reasons to think that the method does provide valid data on behaviour of larval fishes of those species that are amenable (Leis et al. 1996, Leis & Carson-Ewart 1998). For example, larvae feed while being followed, they frequently will swim back toward the divers when predatory fishes approach, they swim at about half of their sustainable speed as determined in laboratory raceways, they do different things in different situations (e.g., swim in a different direction or a different depth in location x than in location y), and when settling, they carefully examine several potential settlement sites before selecting one upon which to settle. Larvae are released in randomly-chosen directions into the ocean by divers to avoid any bias to directionality. The larva selects the direction of travel, and the divers then follow 1-2 m behind and at the same level to avoid shading the larva.

For the study of orientation, a direct comparison of following methodology with the drifting *in situ* chamber (DISC, which is a mesh-enclosed larva-containing chamber drifting in the ocean suspended in mid water from a float, and which includes a camera to record orientation behaviour of the larva within the chamber – see Paris et al. 2008, Irisson et al. 2009) found that both methods returned the same results (Leis et al. 2014). This comparison corroborates the orientation results from diver following. It also alleviates concerns about the noise that SCUBA divers make while following larvae, as divers and their noise are absent in DISC deployments. Further, a study of the effects of

broadcast sound on orientation behaviour using diver following observations showed that larvae *in situ* could hear the broadcast sounds and distinguish among different sounds in the presence of divers (Leis et al. 2002).

The diver following methodology has been used in over 25 published studies to date (for reviews, see Leis 2006, 2010, Leis et al. 2011). It has become an accepted, standard means of studying behaviour of fish larvae in the ocean, and has helped overturn the paradigm of the feeble larval fish drifting passively with the currents (Leis 2015).

LITERATURE CITED

Hindell JS, Jenkins GP, Moran SM, Keough MJ (2003) Swimming ability and behaviour of post-larvae of a temperate marine fish re-entrained in the pelagic environment. Oecologia 135:158-166

Irisson J-O, Guigand C, Paris CB (2009) Detection and quantification of marine larvae orientation in the pelagic environment. Limnol Oceanogr Methods 7:664-672

Leis JM (2004) Vertical distribution behaviour and its spatial variation in late-stage larvae of coral-reef fishes during the day. Mar Freshwat Behav Physiol 37(2):65-88

Leis JM (2006) Are larvae of demersal fishes plankton or nekton? Adv Mar Biol 51:59-141

Leis JM (2010) Ontogeny of behaviour in larvae of marine demersal fishes. Ichthyol Res 57:325-342

Leis JM (2015) Is dispersal of larval reef fishes passive? In: Mora C (ed) Ecology of fishes on coral reefs. Cambridge University Press, Cambridge, U.K., p 223-226

Leis JM, Carson-. BM (1997) *In situ* swimming speeds of the late larvae of some coral reef fishes. Mar Ecol Prog Ser 159:165-174

Leis JM, Carson-Ewart BM (1998) Complex behaviour by coral-reef fish larvae in open-water and near-reef pelagic environments. Environ Biol Fishes 53:259-266

Leis JM, Carson-Ewart BM (2002) *In situ* settlement behaviour of damselfish larvae (Pisces: Pomacentridae). J Fish Biol 61:325-346

Leis JM, Carson-Ewart BM (2003) Orientation of pelagic larvae of coral-reef fishes in the ocean. Mar Ecol Prog Ser 252:239-253

Leis JM, Sweatman HPA, Reader SE (1996) What the pelagic stages of coral reef fishes are doing out in blue water: daytime field observations of larval behaviour. Marine and Freshwater Research 47:401-411

Leis JM, Carson-Ewart BM, Cato DH (2002) Sound detection in situ by the larvae of a coral-reef damselfish (Pomacentridae). Mar Ecol Prog Ser 232:259-268

Leis JM, Hay AC, Trnski T (2006) *In situ* behavioural ontogeny in larvae of three temperate, marine fishes. Mar Biol 148:655-669

Leis JM, Piola RF, Hay AC, Wen C, Kan K-P (2009) Ontogeny of behaviour relevant to dispersal and connectivity in larvae of two non-reef demersal, tropical fish species. Marine and Freshwater Research 60:211-223

Leis JM, Siebeck UE, Dixson DL (2011) How Nemo finds home: the neuroecology of dispersal and of population connectivity in larvae of marine fishes. Integr Comp Biol 51:826-843

Leis JM, Paris CB, Irisson J-O, Yerman MN, Siebeck UE (2014) Orientation of fish larvae *in situ* is consistent among locations, years and methods, but varies with time of day. Mar Ecol Prog Ser 505:193-208

Paris CB, Guigand C, Irisson J-O, Fisher R, D'Alessandro E (2008) Orientation With No Frame of Reference (OWNFOR): A novel system to observe and quantify orientation in reef fish larvae. In: Grober-Dunsmore R, Keller BD (eds) Proceedings of a Special Symposium, 9-11 November 2006, 59th Annual Meeting of the Gulf and Caribbean Fisheries Institute. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Belize City, Belize, p 52-62

Trnski T (2002) Behaviour of settlement-stage larvae of fishes with an estuarine juvenile phase: *in situ* observations in a warm-temperate estuary. Mar Ecol Prog Ser 242:205-214