Embryonic learning of chemical cues via the parents' host in anemonefish (*Amphiprion ocellaris*)

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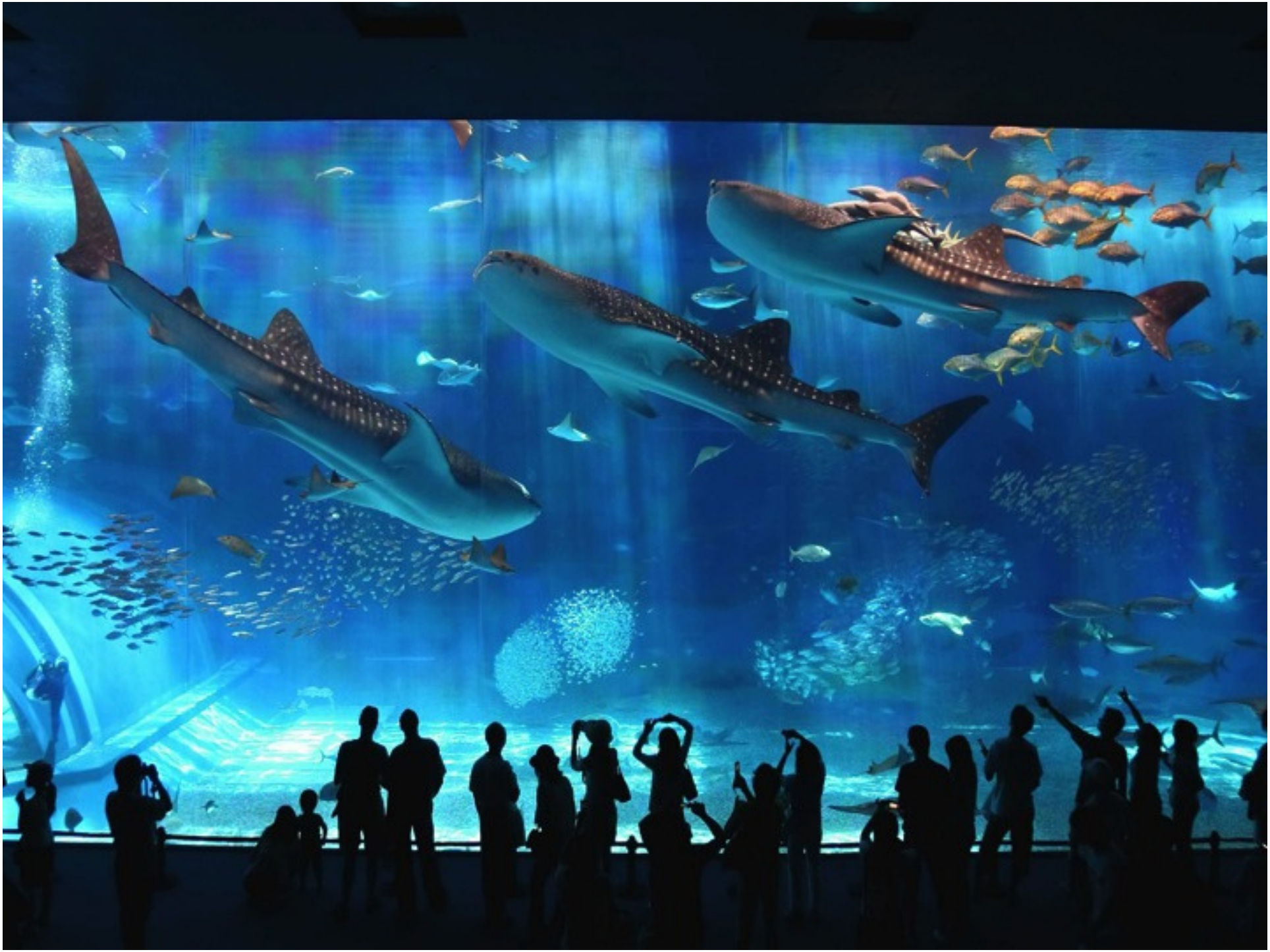


＜Life cycle of anemonefish＞

* Spawning site : immediately adjacent to the column or oral disk of the host anemone
  Eggs are usually touched by the tentacles or body of the host.

* Eggs hatch in about one week, after sunset---->Pelagic life stage

* Become juveniles in about 7～10 days after hatching------>Benthic life stage

Species-specific partnerships of 6 species off the Okinawa Islands

A. ocellaris & Sg

A. perideraion & Hm

A. polymnus & Sh

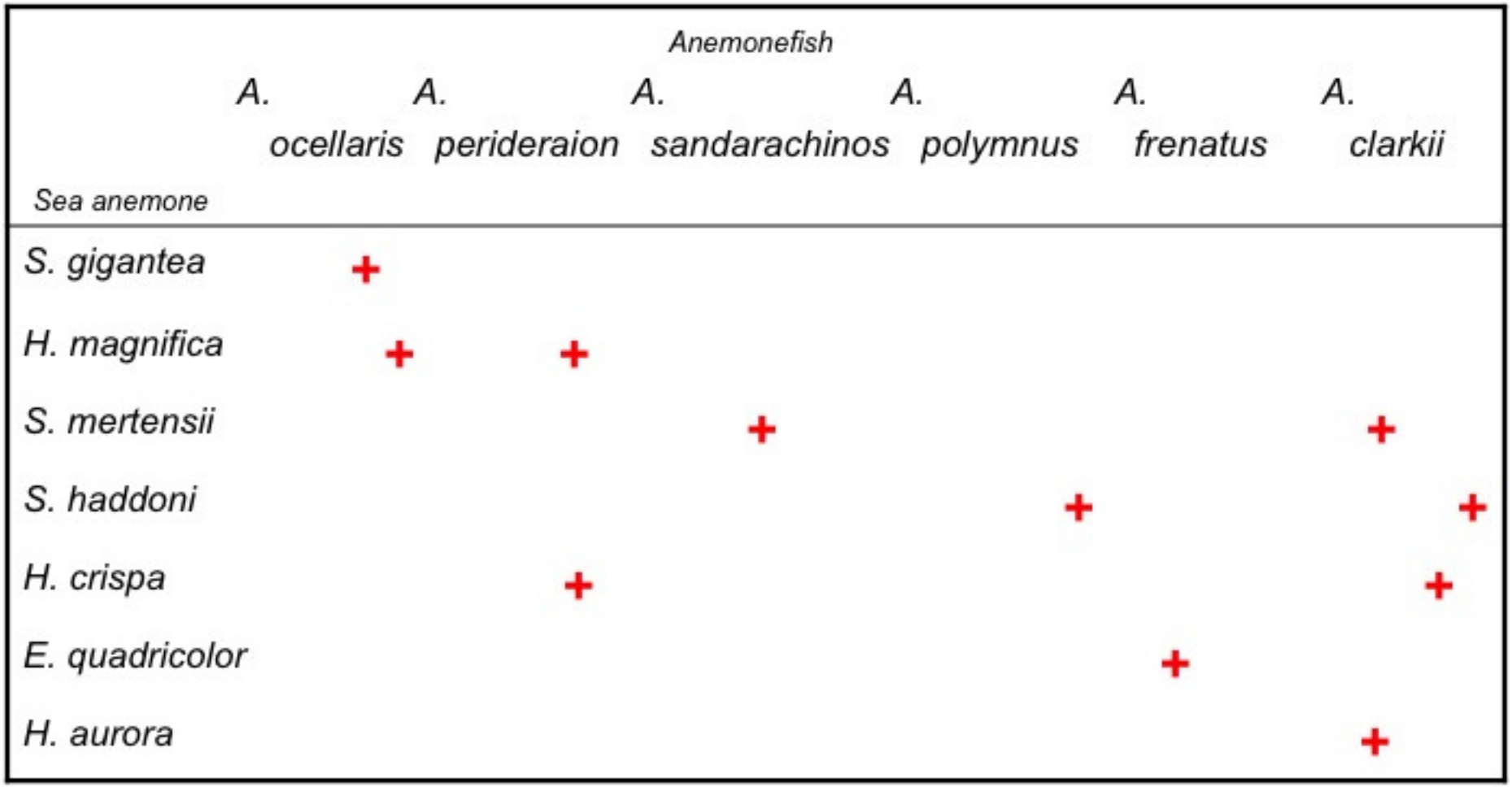
A. sandaracinos & Sm

A. frenatus & Eq

A. clarkii & Hc

Anemonefish juveniles recognise their species-specific host chemically.
(Miyagawa 1989)

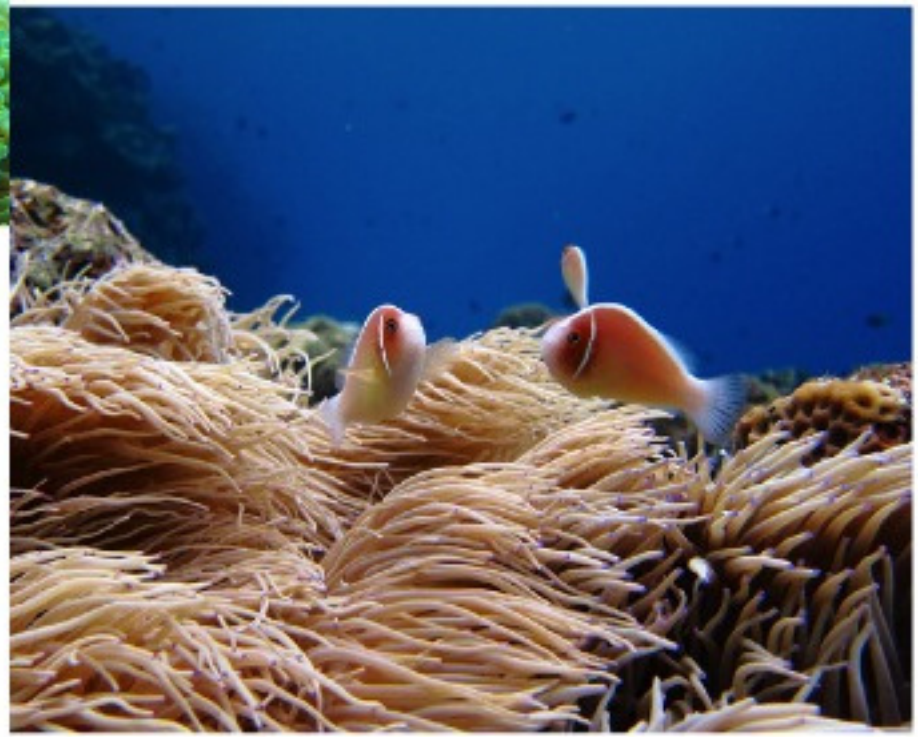
Species-specific partnerships in the coastal region of the Okinawa Islands



Naïve juvenile anemonefish reach their host by recognising chemicals emitted from symbiotic anemones(Miyagawa,1989;Elliott et al.1995)

Typical combination in the Indo-Pacific Ocean
A. perideraion with *Hm*

Rare combination
A. perideraion with *Hc* in Okinawa



Arvedlund & Nielsen (1996) proved the necessity of imprinting via parent's host for the chemical host-recognition of *A.ocellaris juveniles.*

Several important questions still remained

＜The main objectives of this study ＞

1. To verify the existence of basic innate (genetic) recognition
2. To determine how imprinting and innate recognition work together in the host-recognition system
3. To define the duration of the "critical period"
4. To establish the adaptive function of this imprinting

Sea anemones used experiments

2 symbiotic-partner anemone species of *A. ocellaris*

Heteractis magnifica: Hm

Stichodactyla gigantea: Sg

Non-partner anemone

Stichodactyla mertensii: Sm

Heteractis crispa: Hc

Entacmaea quadricolor: Eq

Fig. 1 Trough Experiment

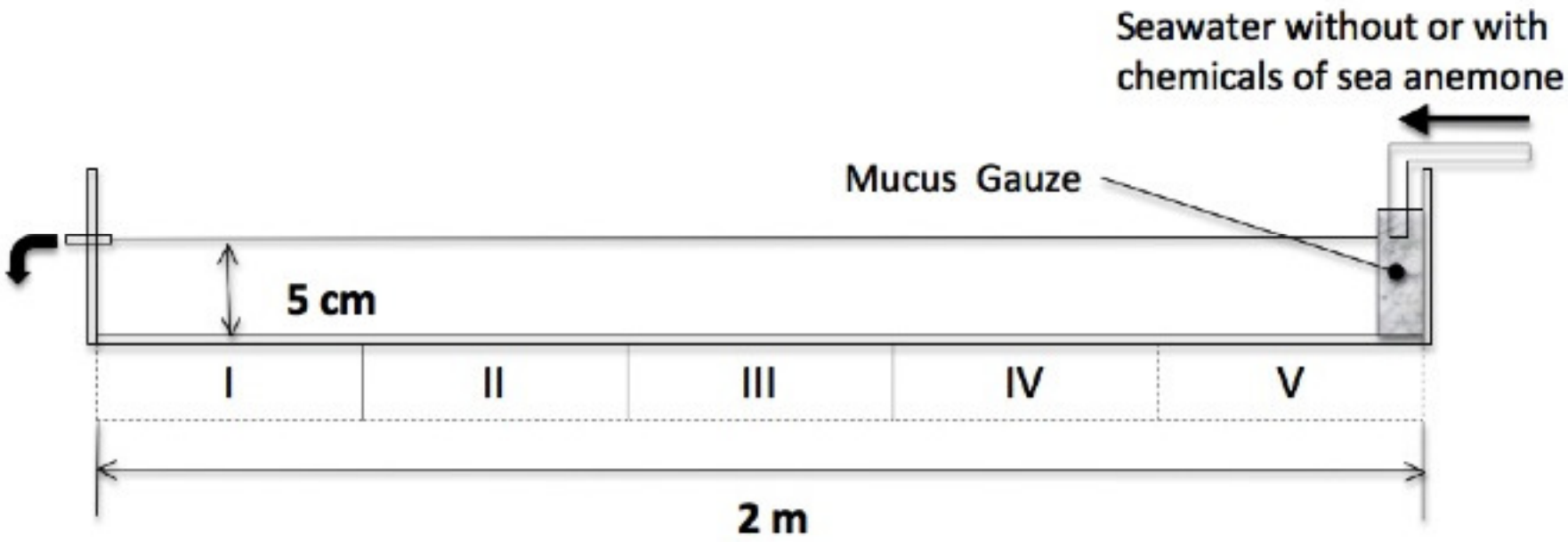


Fig. 2 Example of the average positions of five non-imprinted juveniles during a typical trough experiment over 60 min.

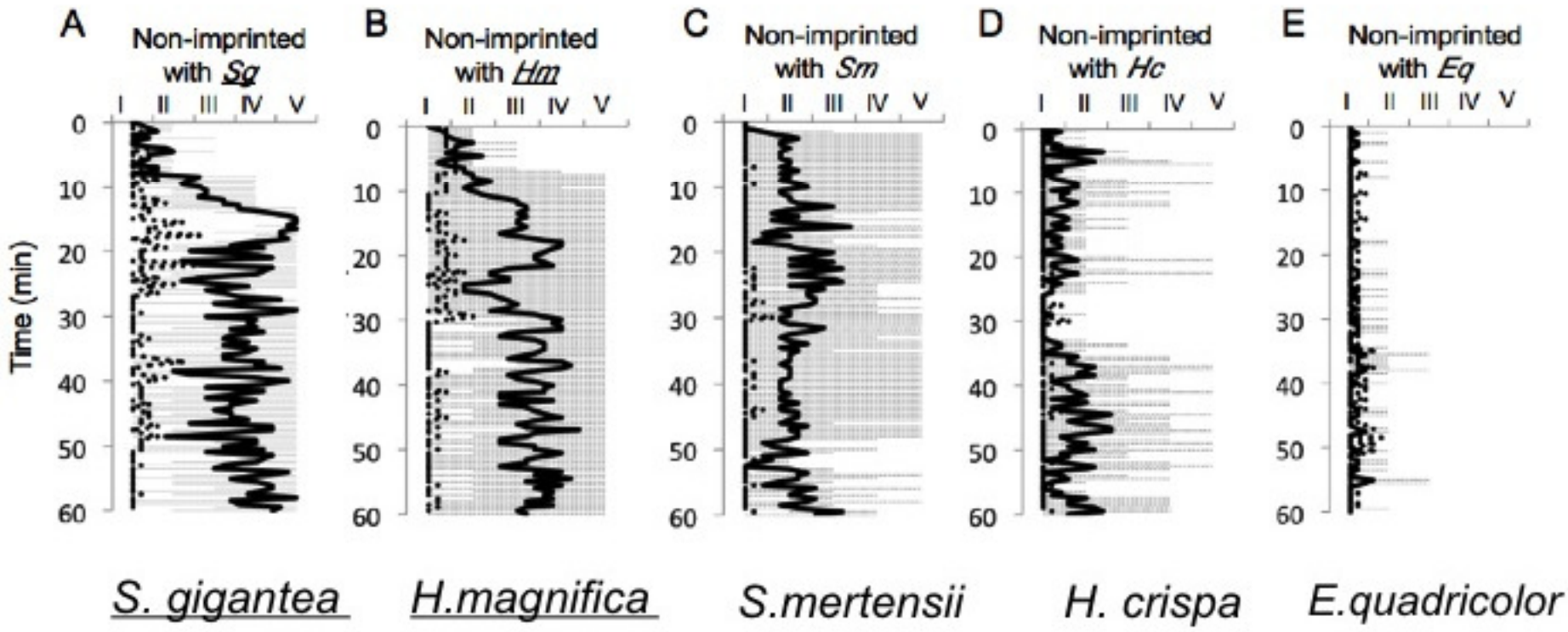
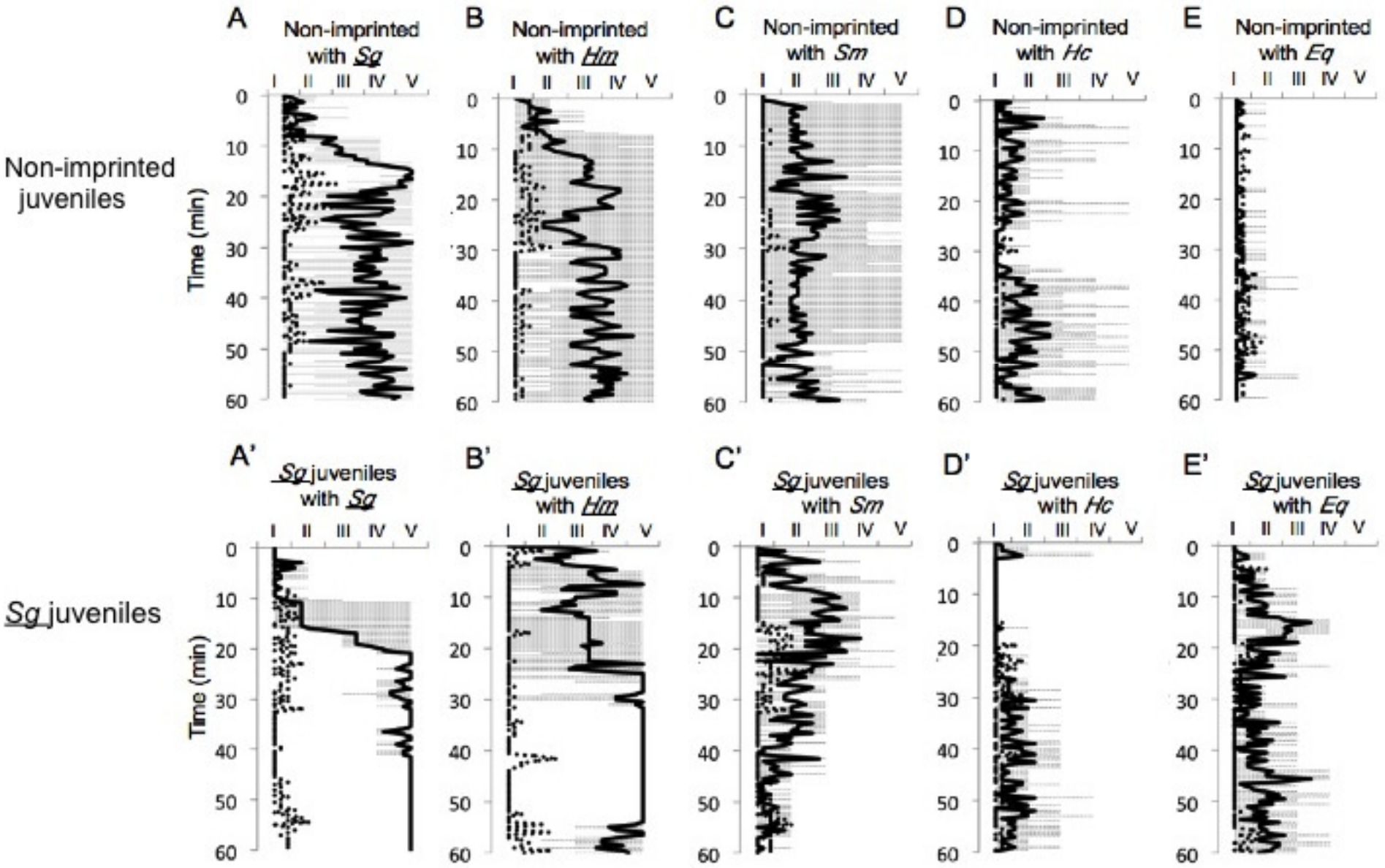


Fig.3

Difference between the results of non-imprinted and imprinted *Sg* juveniles



Imprinted juveniles by symbiotic parents' host:

Almost all tested fish were attracted and stayed in section V.

Imprinted juveniles showed vastly different behaviour from that of non-imprinted juveniles in the following ways:

i) They quickly moved straight to section V where the chemicals of the symbiotic anemone were been poured into the trough

ii) They tended to stay in section V and intimately touch the inlet tube that was introducing the chemicals

Meanwhile, imprinted juveniles lost their weak attraction to non-partner anemone species.

Imprinting via the parents' single host provided a sufficient cue for reaching the two host species.

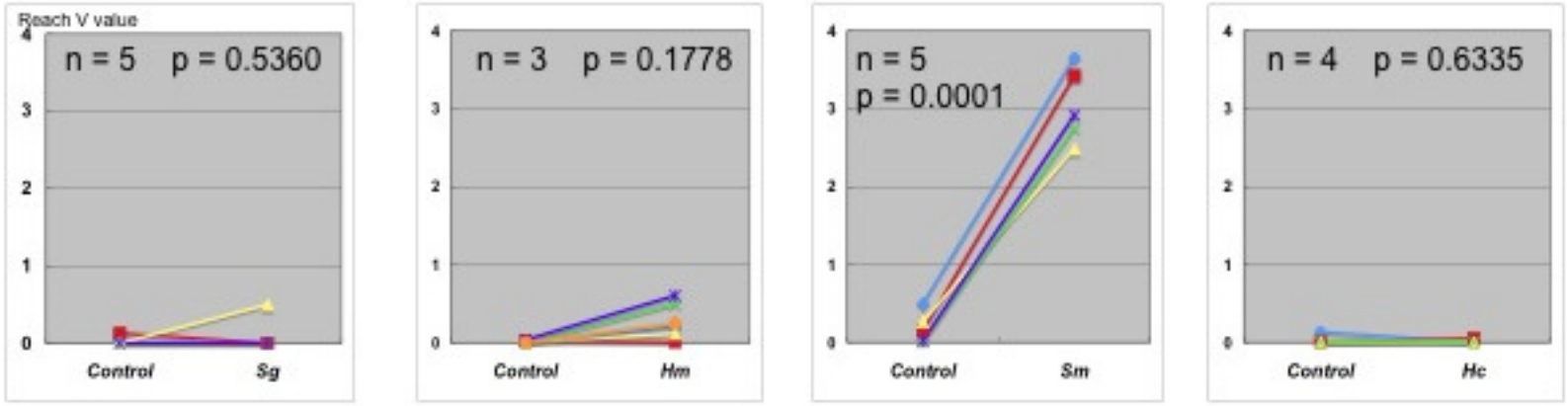
Imprinting via parents' host complements the innate recognition, leading to rigid species-specific recognition.

Why is the weak innate recognition of non-symbiotic anemones programmed？

An *A. ocellaris* pair was made to breed adjacent to *Sm* (Non-partner anemone).

Sm juveniles recognised *Sm*.

On the other hand, recognition of the symbiotic anemones was suppressed.



Sg *Hm* *Sm* *Hc*

Weak innate recognition of non-symbiotic anemones is likely to be “a spare recognition” for adapting to some environmental situations:especially in the case of host shortage due to interspecific competition among sympatric species over common hosts.

The possibilities of learning

Pre-hatching or Post-hatching?

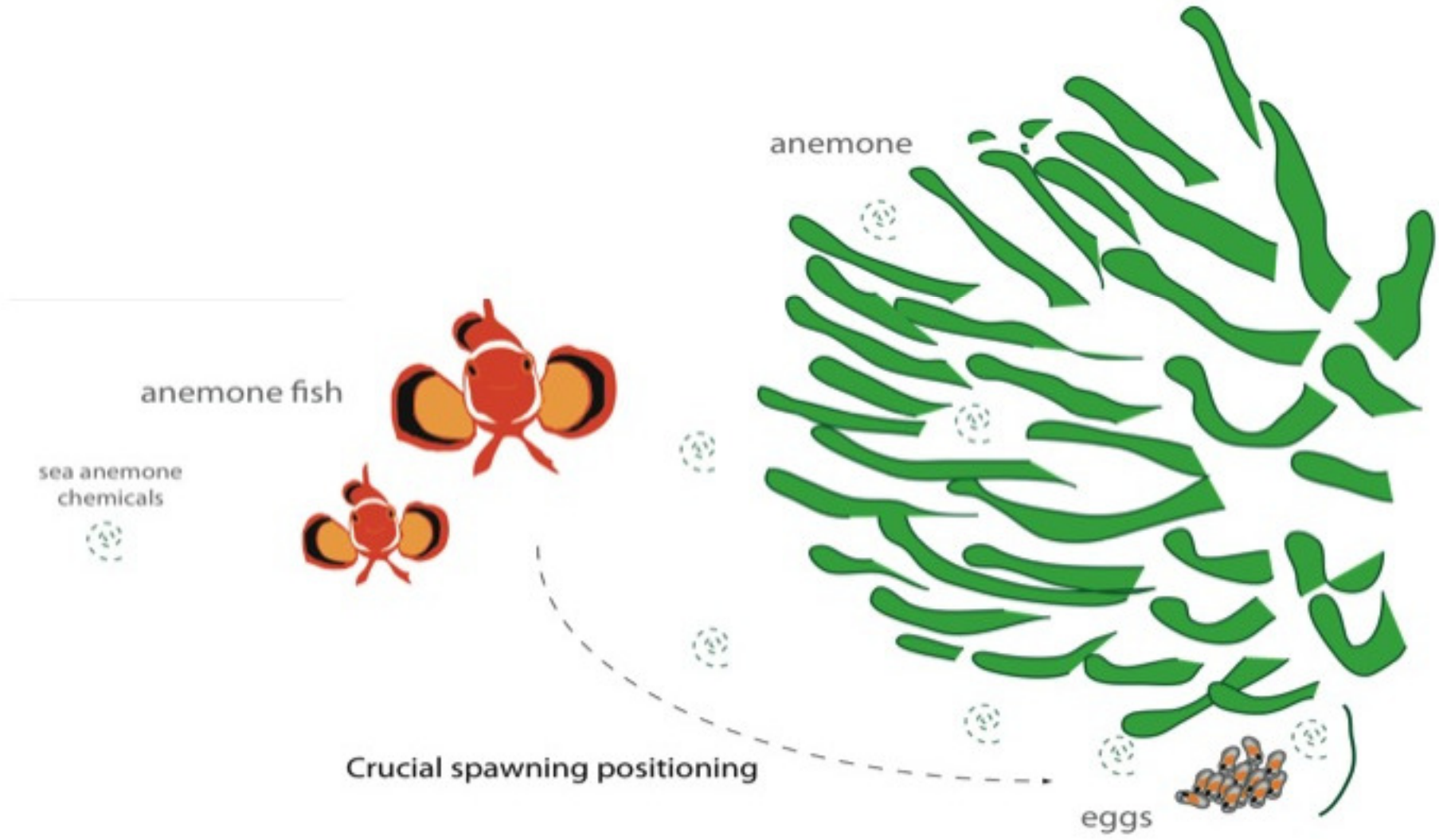
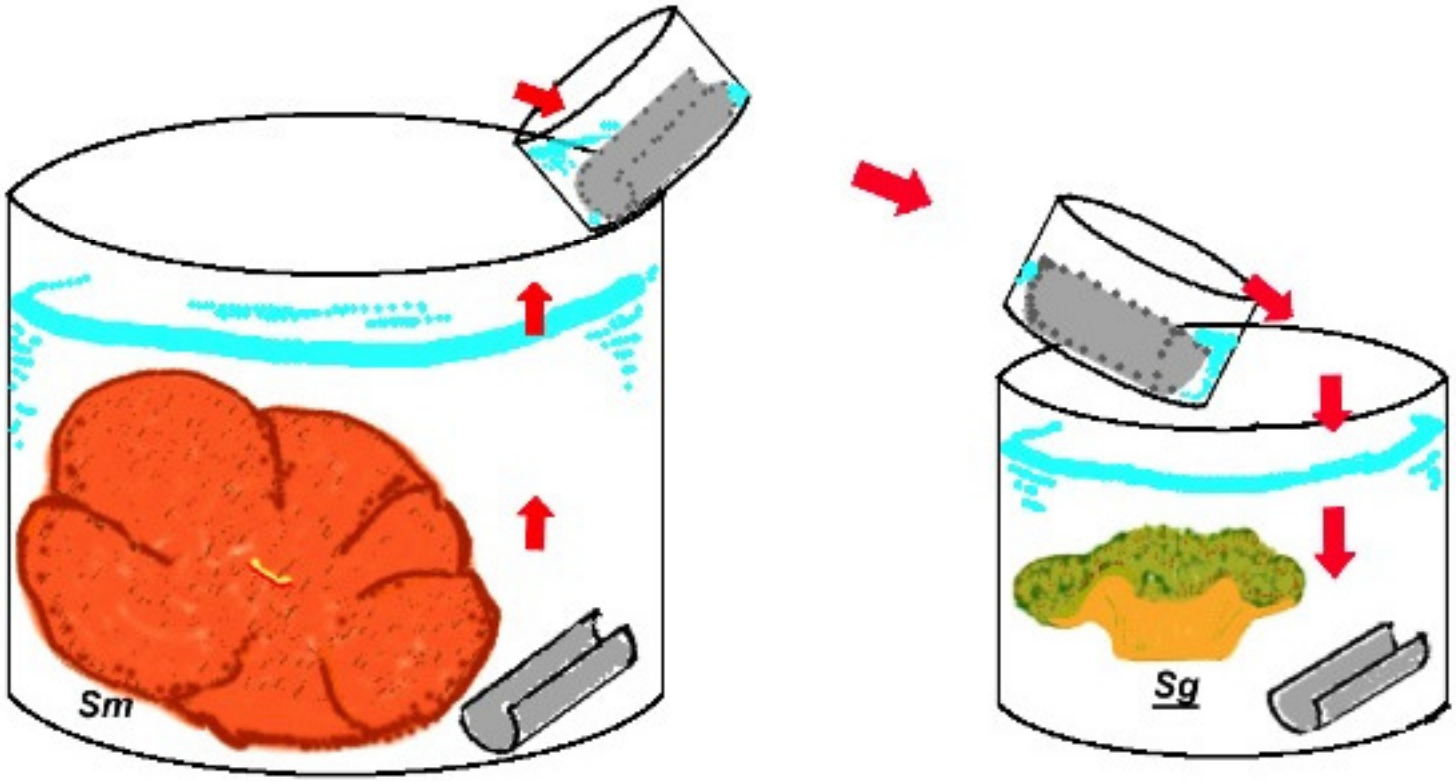


Fig.

Determination of the "Critical period"

Host-exchange Experiment



Results of the host-exchange experiment: imprinted rates by each anemone in S

Hatched condition		Rates of imprinted juveniles (%) 1st experiment 2011/08/01	Rates of imprinted juveniles (%) 2nd experiment 2011/10/05
Before transferring (hatched in *Sm* tank)		(N=45)	(N=18)
	Sm juveniles	26.7	11.1
	Sg juveniles	0	0
	Non-imprinted	73.3	88.9
After transferring (hatched in *Sg* tank)		(N=84)	(N=64)
	Sm juveniles	45.2	14.1
	Sg juveniles	19.1	23.4
	Non-imprinted	35.7	62.5

N= number of tested juveniles in each tank.

Conclusion

1. Imprinting via parents' host complements the innate recognition, leading to species-specific host-recognition.
2. When combined with imprinting, innate recognition of non-partners serves to supplement the recognition of those species.
3. The critical period starts from pre-hatching until immediately post-hatching.

Imprinting via the parents' host helps the next generation obtain clues to reach the most appropriate host species in the local habitat, reflecting the ecological situation of their parents.