

PLoS Biology

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Cleaner wrasse pass the mark test. What are the implications for consciousness and self-awareness testing in animals?

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20 Short title: The mark test in fish

Abstract

22

The ability to perceive and recognise a reflected mirror image as self (mirror self-recognition, MSR) is considered a hallmark of cognition across species. Although MSR has been reported in mammals and birds, it is not known to occur in any other major taxon. A factor potentially limiting the ability to test for MSR is that the established assay for MSR, the mark test, shows an interpretation bias towards animals with the dexterity (or limbs) required to touch a mark. Here, we show that the cleaner wrasse fish, *Labroides dimidiatus*, passes through all phases of the mark test: (i) social reactions towards the reflection, (ii) repeated idiosyncratic behaviours towards the mirror (contingency testing), and (iii) frequent observation of their reflection. When subsequently provided with a coloured tag, individuals attempt to remove the mark in the presence of a mirror but show no response towards transparent marks, or to coloured marks in the absence of a mirror. This remarkable finding presents a challenge to our interpretation of the mark test – do we accept that these behavioural responses in the mark test, which are taken as evidence of self-recognition in other species, mean that fish are self-aware? Or do we conclude that these behavioural patterns have a basis in a cognitive process other than self-recognition? If the former, what does this mean for our understanding of animal intelligence? If the latter, what does this mean for our application and interpretation of the mark test as a metric for animal cognitive abilities?

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42 **Introduction**

The mark test, in which a coloured mark is placed on a test subject in a location that can only
44 be viewed in a mirror reflection, is held as the benchmark behavioural assay for assessing
whether an individual has the capacity for self-recognition [1,2]. In human infants,
46 approximately 65% of individuals pass the mark test by 18 months of age by touching the
mark with their hands while viewing their reflection [3], although some individuals pass earlier
48 and some never pass. Accumulating reports claim that many other animal species also pass
the mark test, including chimpanzees [1], elephants [4], dolphins [5,6] and corvids [7], while
50 many other species are apparently unable to pass the test [8; but see 9-11]. Nevertheless,
the interpretation of these results is subject to wide debate, and the certainty with which
52 behavioural responses during the mirror test can be taken as evidence of self-awareness in
these animals is questioned (8,12,13). This problem is exacerbated when the taxonomic
54 distance increases between the test species and taxa for which the test was initially
designed. Can for example the behavioural results recorded for primates during the mirror
56 test be meaningfully compared with those in birds? If yes, does this mean a bird that passes
the mirror test is self-aware? More generally, if we are interested in understanding and
58 comparing cognition and problem-solving across taxa, can we assume that equivalent
behaviours represent equivalent underlying cognitive processes? With particular reference to
60 the mark test, here we explore what forms of behaviour in fish could be taken as evidence of
self-awareness, as has been done for primates and other taxa.

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Given that the mark test as designed for humans and primates relies on hand gestures
64 toward the marked region and changes in facial expression, we also ask whether it is even
possible to interpret the behaviour of 'lower' taxonomic groups during the mark test in the
66 same way as for 'higher' taxa. If not, we must ask how useful the mark test is as a test for
self-awareness in animals. To explore these questions, we here test whether a fish, the
68 cleaner wrasse *Labroides dimidiatus*, displays behavioural responses that constitute passing

the mark test. We then ask what this may mean for our understanding of self-awareness in
70 animals and our interpretation of the test itself.

72 To date, no taxon outside of birds and mammals has passed the mark test. This is despite
many species in other vertebrate classes, such as fish, showing sophisticated cognitive
74 capacities in other tasks [14-17], including transitive inference [18,19], episodic-like memory
[20], playing [21], tool use [22,23], prediction of the behaviour of others by using one's own
76 experience during coordinated hunting [24, 25], cooperating to warn about predators [26,27]
and cooperative foraging [28]. These studies reveal that the perceptual and cognitive abilities
78 of fish often match or exceed those of other vertebrates [15,17], and suggest the possibility
that the cognitive skills of fish could more closely approach those found in humans and apes
80 [14,16,17,24,28].

82 Nevertheless, it can be challenging to employ standardised cognitive tests across species
when performance in the test depends on specific behavioural responses that are not
84 present in all taxa. This may be considered the case for the mark test, which has been
designed to suit the behavioural repertoire of humans and primates [1,2]. Animals that
86 cannot directly touch the marks used in MSR tests, such as fish as well as dolphin, are
therefore regarded as poor test candidates [2,5,29], making direct comparison of their
88 cognitive capacities with those of other vertebrates challenging [30-33]. Although no mark
tests were performed, behavioural observation of manta rays (Chondrichthyes) on exposure
90 to a mirror suggests that these fish show self-directed behaviour toward their reflection [34],
though these results are contested [35,36]. This controversy highlights the need to question
92 what type of behavioural response would be taken as evidence of interacting with the mark in
an animal with the morphology of a ray, and whether these behaviours may have an
94 alternative function, e.g. in social communication. To make a comparison across taxa, one
must carefully consider the inherent biology of the focal species, preferably choosing a
96 species with perceptual abilities and a behavioural repertoire that i) allow it to respond to

coloured marks placed on the body (this is not a given when the sensory systems of animals
98 differ so greatly) and ii) do so in a manner that can be interpreted by a human observer.

100 The cleaner wrasse, *Labroides dimidiatus*, is such a species, forming mutualistic
relationships with larger client fish by feeding on visually detected ectoparasites living on the
102 skin of the clients [37]. Therefore, the cleaner wrasse has sensory and cognitive systems that
are well-equipped for visually detecting spots of unusual colour on the skin surface, as well
104 as the behavioural repertoire required to respond to marks. This species is highly social,
interacting with the same individuals repeatedly over long periods of time, and has
106 sophisticated cognitive abilities, including tactical deception [38-40] and reconciliation [41],
and it can also predict the actions of other individuals [41,42]; these are traits requiring
108 mental abilities that may be correlated with the ability for self-recognition, as seen in other
species [16,29,43-45].

110
During the mirror test, animals must visually locate a mark in a mirror image. The
112 interpretation of the test is that animals regard the mark on the body as unusual and thus
examine it. It is reasonable to predict the wrasse will perceive the coloured marks as visually
114 similar to ectoparasites, and may thus evoke an attentional response that may culminate in a
removal attempt [46-47]. However, lacking hands and arms, any attempt to remove or
116 interact with the mark would necessarily take a different form to that observed for primates or
elephants. Fortunately for the question at hand, many fish taxa, including cleaner wrasse,
118 display a characteristic behaviour that functions to remove irritants and/or ectoparasites from
the skin surface [48,49], termed glancing or scraping. This behaviour may therefore be
120 considered as self-directed, just as for some mammal species that also lack hands in which
scraping is taken as an indicator of self-directed behaviour during the mirror test [29,50]. We
122 therefore consider the cleaner wrasse to possess the necessary sensory biology and
behavioural repertoire to adequately employ the mirror test, and here use a modified
124 experimental paradigm established for studies of humans and apes to test for mirror self-

recognition in a fish. Importantly, this species allows us to ask whether the criteria that are
126 accepted as evidence for mirror self-recognition in mammals and birds can be applied to
other taxa, and if they fulfil these criteria, what it means for our interpretation of the test itself.

128

In applying the mirror test, transitions among three behavioural phases after initial exposure
130 to a mirror are typically [1,4,5,6]; these transitions among behavioural phases are interpreted
as additional evidence of self-recognition, although in themselves do not constitute passing
132 the mirror test [1,4]. We first tested whether the cleaner wrasse passed through all three
behavioural phases upon exposure to a mirror placed in an experimental tank (Fig. 1A), and
134 if so, we describe the phases in cleaner fish. The first phase (*i*) is a social reaction towards
the mirror, apparently as a consequence of the reflection being perceived as an unknown
136 conspecific. In phase (*ii*), animals begin to repetitively perform idiosyncratic behaviours that
are rarely observed in the absence of the mirror. These behaviours are interpreted as
138 contingency testing between their own actions and the behaviour of the reflection [e.g. 1,4].

In phase (*iii*), the animal begins to gaze and examine their reflection as if it is a
140 representation of the self, and uses the mirror to explore their own body in the absence of
aggression and mirror-testing behaviour [1,4,5]. If they passed these phases, we applied the
142 mark test.

144 **Results and discussion**

146 Progression of behaviours in response to the mirror

148 Prior to starting the experiments, the focal fish swam around the tank and showed no
unusual reactions to the covered mirror. Immediately after initial exposure to the mirror, 7 of
150 10 fish responded aggressively to their reflection, attacking it and exhibiting mouth-to-mouth
fighting [45,46] (Fig. 1A,B, Supplementary Movie S1) suggesting that the focal fish viewed
152 the reflection as a conspecific rival. The frequency of mouth fighting was highest on day 1,

and decreased rapidly thereafter, with zero occurrences by day 7 (Fig. 1Ca; cf. with the
154 similar decrease in aggression seen in chimpanzees, and shown in Fig. 2 of [1]) and hardly
any aggression over the following month.

156

As mouth fighting towards the mirror reflection decreased, the incidence of unusual and
158 atypical behaviours (e.g. 'upside-down approach' and 'dashing along mirror'; Table 1, Movies
S2, S3) significantly increased and was highest on days 3–5 (Fig. 1Ca). On days 3 and 4, the
160 estimated average frequency of these atypical behaviours among the seven individuals was
36 times per hour during the daytime. Each of these atypical behaviour types was of short
162 duration (≤ 1 s), often consisting of rapid actions that occurred suddenly within 5 cm of the
mirror. At the end of each movement, the fish remained near the mirror, and appeared as if
164 they were viewing their reflection (Movies S2, S3). These atypical behaviours could be
loosely grouped into five types: not against the mirror reflection, dashing along the mirror
166 without and with attaching the head on the surface (atypical behaviours, *a* and *b*,
respectively); dashing towards the reflection but stopping before touching it (*c*); and
168 idiosyncratic postures and actions of short duration performed in front of the mirror: upside-
down approach (*d*), and quick dance (*e*) (Table 1). While it is possible to interpret these
170 behaviours as a different form of aggression or social communication, they have not been
recorded in previous studies of social behaviour in this species [46] and were not part of a
172 courtship display, as all of the subject fish were females.

174 These atypical behaviours were highly repeatable within an individual, with each fish
performing one or two types of behaviour more than 400 times a day on average during days
176 3 and 4 (Table 2; Fisher's exact probability test for count data with simulated *P*-value based
on 2,000 replicates of $P = 0.0005$). Crucially, these behaviours occurred only upon exposure
178 to the mirror, and were not observed in the absence of the mirror (i.e. before mirror
presentation). Almost all of the behaviours ceased by day 10 (Fig. 1Ca), and thereafter were
180 hardly observed at all over the following month.

182 These behaviours are different from the previously documented contingency-testing
behaviours of great apes, elephants and magpies [1,4,7], but given the taxonomic distance
184 between them, this could hardly be otherwise. While primates and elephants may perform
more anthropomorphic behaviours such as changing facial expression, or moving the hands,
186 legs or trunk in front of the mirror, wrasse and other fishes cannot perform behaviour that is
so easily interpreted by a human observer. Nevertheless, behaviours such as rapid
188 swimming and other spontaneous actions could represent alternative indices of contingency
that are within the behavioural repertoire of the study species (Table 1).

190

In summary, the atypical movements observed in cleaner wrasse were characterised by
192 almost every aspect of contingency-testing behaviour documented previously [1,4,5,7]: 1)
atypical and idiosyncratic, 2) occurring repeatedly, 3) occurring in front of a mirror, 4) not
194 occurring in the absence of a mirror, 5) occurring after a phase of initial social behaviour, 6)
occurring over a short period of time and 7) distinct from aggressive behaviour. Fulfilment of
196 these conditions supports the contingency-testing hypothesis. Although we reserve
judgement as to whether these behaviours should be interpreted as evidence that the fish
198 examine and perceive the reflection as a representation of self, we nevertheless conclude
that these behaviours are consistent with phase (*ii*) of MSR as presented for other taxa.

200

In phase (*iii*), which is difficult to clearly distinguish from phase (*ii*), species that pass the
202 mark test increase the amount of time spent in front of the mirror in non-aggressive postures
while viewing the mirror image [1,4,5,7]. This interpretation is again rife with pitfalls, as it
204 requires an assessment of the intentionality of unusual animal behaviours. An agnostic
approach is to simply measure the amount of time animals spend in postures that reflect the
206 body in the mirror [2]. This gives an upper measurement of the time in which animals could
be observing their reflection while making no inferences about the intentionality of the act.
208 We observed an increase in the amount of time spent in non-aggressive postures while close

to the mirror (distance of < 5 cm), peaking on day 5 after mirror presentation and remaining
210 consistently elevated (Fig. 1Ca; $107.0 \text{ sec} \pm 21.2 \text{ [SD]}/10 \text{ min}$) versus days 1–4 and the
several days prior to mirror presentation ($37.0 \text{ sec} \pm 11.5$, Wilcoxon sign-ranked test, $T = 36$,
212 $P = 0.008$); this behaviour was consistent with phase (iii) of MSR. We did not observe
specific viewing behaviour that is seen in chimpanzees and elephants, e.g. trying to look at
214 body parts, such as inside the mouth or between the legs. It is inherently difficult to
distinguish such looking behaviours from other behaviours primarily because gaze direction
216 could not be determined in this species. Technological developments that allow eye tracking
in free-swimming fish may alleviate this difficulty in future studies.

218

Similar to other studies, not all individuals we tested passed through each phase of the test.
220 After the initial presentation of the mirror, three fish (#4, #5, #6) showed low levels of
aggression and rarely performed atypical behaviours during period E1 (Fig. 1Cb). Instead,
222 these three individuals spent relatively longer periods in front of the mirror, as is typically
observed during phase (iii) in other focal fish (Fig. 1Cb). By applying the same criteria as
224 applied for other instances of the test, we conclude these fish failed the test. However, an
alternative explanation is that these fish had already passed through the initial phases; at the
226 start of the experiment, the glass wall on the opposite side of the mirror in the tanks of these
three fish was slightly reflective due to differences in lighting in the room, and the focal fish
228 were observed to occasionally remain in front of the glass wall. These observations suggest
that these three fish may have already passed through phases (i) and (ii) during the
230 acclimation phase before the start of experiment. As discussed below, these three fish
exhibited good responses to the mark test.

232

Species with MSR distinguish their own reflection from real animals viewed behind glass
234 [e.g. 29]. When we exposed naïve cleaner wrasse to conspecifics behind glass, we observed
fundamentally different responses towards their mirror image. Aggressive behaviour
236 frequency towards real fish was generally low, yet did not diminish appreciably during the 2-

week testing period (Fig. 1D). Time spent within 5 cm of the glass in the presence of
238 conspecifics was also higher than that in the presence of the mirror. Importantly, no atypical
or idiosyncratic behaviour (i.e. contingency-testing) was exhibited towards conspecifics.
240 These behaviours were only observed upon exposure to the mirror.

242 Mark test

244 In the second part of the experiment, we used a modified standard mark test protocol to
assess reactions to visible or sham (clear or un-pigmented) coloured marks. We used
246 subcutaneously injected elastomer (see Material and methods; Northwest Marine
Technology Inc. (NMTI), Anacortes, WA, USA). The coloured marks are applied with a small
248 amount of coloured gel using a fine needle, a procedure, which has been repeatedly shown
not to affect fish behaviour [51-54, NMTI], and is widely used in fish behavioural studies.
250 Moreover, use of both visible coloured and invisible sham marks directly controlled for any
effects of application, including irritation or tactile sensations around the marking site.
252 Nevertheless, the procedure was necessarily modified from terrestrial tests due to the
requirements of provisioning marks in the aquatic environment, and on animals covered in a
254 protective mucus coating. Because of this, it involved potentially higher tactile stimulation
than e.g. paint marks on elephant skin. We controlled for these direct tactile effects with a
256 sham-marking experimental phase, observing no effect (see below). Nevertheless, we must
consider a recent study that showed visual-somatosensory training induced self-directed
258 behaviour in rhesus monkeys [10,11], which could not be achieved through visual stimuli
alone. Our study differs in that we do not provide direct somatic stimulation during the mirror
260 test, and much evidence suggests that no further tactile stimulation is maintained by
elastomer injections after the procedure. However, given the nature of the mark application,
262 we cannot rule out that a combination of visual and tactile cues produces the behavioural
responses we describe.

264

Fish were marked at night while under anaesthesia, and they swam normally early the next
266 morning under a no-mirror condition (Kohda, pers. obs.). After the initial settlement period
“E1” (i.e. the initial 2 weeks of phases *i–iii*), we evaluated behaviour during periods “E2” (no
268 mark), “E3” (injection with invisible sham mark), “E4” (injection with coloured mark with no
mirror present) and “E5” (coloured mark with mirror present) during a further 2-week period.
270 The sham and coloured marks were applied on the right side of the head of two fish, on the
left side of the head of two other fish and under the throat in a further four fish (Fig. 2A);
272 these areas were only visible in the mirror. Each mark was in the form of a small brown dot
resembling an ectoparasite.

274

1) Viewing coloured marks in the mirror

276

We first examined whether fish were assuming postures in front of the mirror that would
278 reflect the marked site by categorising all body postures performed within 5 cm of the mirror
into three types: postures exposing the right side of the head to the mirror, postures exposing
280 the left-side of the head, and frontal-vertical postures exposing the head, throat, and
underside to the mirror. These postures would respectively reflect the right face mark, the left
282 face mark, and the throat mark (Fig. 2B). We predicted that if fish were attempting to observe
the coloured marks on body parts reflected in the mirror, they would assume postures that
284 facilitated this observation of the mark significantly more frequently during E5 (mirror, colour
mark), than E2 (mirror, no mark), or E3 (mirror, transparent sham mark). Two independent
286 and comprehensive analyses of the videos were conducted (by MK, JA), as well as two
further blind analyses by unrelated researchers of a subset (15%) of the videos; the
288 frequencies of the postures were highly correlated between the analyses ($r = 0.988$).

290 Posturing behaviours against the marked sites during periods E2 and E3 were infrequent and
were not different between the two periods (Fig. 2Ca), a pattern driven by all fish except fish
292 #7, which showed equal distributions of viewing angles (Table 3). This shows that the

marking procedure itself had minimal effect on fish behaviour. In contrast, time spent
294 posturing while viewing the marked sites was significantly higher in the coloured- (E5) versus
no- (E2) and sham-marked (E3) periods (Fig. 2Ca), as predicted. This pattern held for all
296 individuals except fish #2, regardless of the sites marked (Table 3). Note that no
comparisons to E4 can be made with respect to observations of reflections, as no mirror was
298 present during that period. Moreover, the time spent in postures reflecting the two remaining
unmarked sites (e.g. right side of head and throat, for a fish marked on the left side of head)
300 for each fish were not different among periods (Fig. 2Cb). Taken together, these findings
demonstrate that cleaner wrasse spend significantly longer in postures that would allow them
302 to observe colour-marked sites in the mirror reflection. These reactions also demonstrate that
tactile stimuli alone are insufficient to elicit these behaviours, as they were only observed in
304 the colour mark/mirror condition. Rather, direct visual cues, or a combination of visual and
tactile stimuli, are essential for posturing responses in the mirror test. In previous studies on
306 dolphins, similar patterns of activity were considered to constitute self-directed behaviour [5].

308 2) Scraping of the colour-marked throat after viewing it in the mirror

310 Although they cannot touch their own bodies directly, many species of fish scrape their
bodies on a substrate to remove irritants and/or ectoparasites from the skin surface [48,49].
312 When we marked fish with brown-pigmented elastomer on the lateral body surfaces in
locations that could be viewed directly, the fish increased scraping behaviour of the mark
314 sites, indicating they regard the colour dots as ectoparasites to be removed (Supplementary
Figure S1). Similar scraping of colour-marked areas during the mark test is interpreted as an
316 indicator of self-directed behaviour for some mammal species that do not have hands
[29,50]. Accordingly, we hypothesised that the cleaner wrasse would scrape their bodies in
318 an attempt to remove coloured marks from body parts not directly visible after observing
them in the mirror (and crucially, that they did not scrape invisible sham marks, nor coloured
320 marks in the absence of a mirror). As discussed earlier, if we observe a behaviour in a fish

that is accepted to be functionally equivalent to a similar behaviour in mammals (in this case
322 scraping), and that behaviour is accepted as being self-directed in those mammals [29, 50],
then it raises the question whether this behaviour may be similarly considered self-directed in
324 the fish. If this position is accepted, then any scraping behaviour of coloured marks in the
mirror condition would constitute compelling evidence that fish use mark-directed behaviour
326 to remove visually perceived coloured marks from their bodies. By extension and comparison
to similar mirror test studies, this would raise the question of whether fish are therefore aware
328 that the mirror reflection is a representation of their own body.

330 Like many natural behaviours, some scraping of the body flanks was observed outside the
mirror condition in our studies. This body scraping behaviour was also difficult to distinguish
332 from head scraping. Because of these factors we took throat scraping, and not face scraping,
as the only evidence of a putative self-directed behaviour because it was never observed
334 outside the period E5 in any of the subject fish. It is also important to note that fish marked
on the head laterally scraped the body flank/facial region, but never the throat region, during
336 period E5, providing further evidence that marking itself does not induce throat scraping.
Three of the four throat-marked fish frequently scraped their throats against the substrate
338 after being exposed to the mirror during period E5 (Fig. 3A, B, Movies S4–S6), but none of
the four fish exhibited this behaviour during E2–E4. We observed 37 separate instances of
340 throat scraping during E5 (15 for fish #1, 16 for fish #4, 6 for fish #21; Friedman test, $\chi^2 = 9.0$,
 $df = 3$, $P = 0.029$; binomial test within individuals, E2, E3 and E4 vs. E5: 0 vs. 15 scrapings,
342 $P < 0.0001$ in fish #1, 0 vs. 16 scrapings, $P < 0.0001$ in fish #4, 0 vs. 6 scrapings, $P = 0.031$
in fish #21). These three fish attempted to scrape their throats but this was occasionally
344 executed awkwardly, probably because they were not accustomed to performing this
behaviour. As the marks were identical in periods E4 and E5, with the only change being the
346 visibility of the mirror, the difference in throat scraping provides further strong evidence that
the colour injection itself did not cause direct physical stimulation that would lead to the
348 observed behaviours (e.g. itching or pain).

350 These results accord with the increased amount of time spent in postures indicating
observation of the coloured marks in the reflection only during period E5 (Fig. 2Ca). The
352 motivation for scraping the mark is potentially to remove a perceived ectoparasite, which
these wild-caught fish would have experienced previously. In all cases ($n = 37$), the scraping
354 behaviours followed soon after the fish had assumed a posture that reflected the throat mark,
with an average latency between observation in the mirror and scraping of the substrate of
356 $1.93 \text{ sec} \pm 1.16$ ($n = 37$; see Fig. 3C, Movies S4–S6). However, posturing was not always
followed by scraping. The physical substrate on which fish scraped varied among individuals:
358 all scraping was done in a narrow area of the sandy bottom by fish #1, and all and the
majority (14/16) of the scraping of fishes #21 and #4 was done on a rock in the corner of the
360 tank despite the same arrangement in all tanks. This may be because fish learn successful
scraping techniques associated with specific substrates and continue to use them.

362

The majority of the throat scraping behaviour was immediately followed by another frontal-
364 vertical posture performed in front of the mirror (after $1.82 \text{ sec} \pm 1.46$; $n = 31$; Movie S6).
Assuming frontal-vertical postures during swimming is atypical, and in these postures
366 observation of the marked site via the mirror-reflection is possible. As such, this pattern of
behaviour may constitute contingency testing, in this case to check if the perceived parasite
368 had been removed by the scraping attempt. While this interpretation does imply intentionality
on the part of the animal, the general rarity of this behavioural sequence, and frequency with
370 which it was displayed during the mark test, provide compelling evidence for this
interpretation. Indeed, this type of behaviour is similar to that of chimpanzees, which examine
372 and smell their fingers after touching a paint mark [1,8], and which is considered intentional
self-directed behaviour in that species.

374

Three of the four throat-marked individuals in this study passed the mark test, a success ratio
376 comparable to other species tested previously; one of three Asian elephants passed the test

[4], as did two of five magpies [6]. Fish #20 in our study was throat-marked but did not
378 perform throat scraping (Fig. 3B). However, this fish exhibited intensive contingency-testing
behaviours (a) and (b) during period E1, prior to colour-marking, similar to the other fish
380 (Table 2), and assumed postures (self-directed behaviour) that reflected the throat more
frequently during E5 after colour marking (Table 3). According to the mark test criteria used
382 for dolphins [5], these results suggest that this wrasse recognised the reflection as self, but
“fell at the last hurdle”. Nevertheless, given the controversial nature of the mark test in non-
384 primates, and questions over the interpretation of these behaviours [8], we do not take this
result as conclusive evidence for MSR in this individual. We do point out, however, that by
386 the same criteria used for e.g. dolphins, we would conclude that all four throat-marked fish
recognised themselves in the mirror.

388

In this study we applied the mark test, a controversial assessment of animal cognition [8], to
390 a fish, a taxonomic group often considered to have lower cognitive abilities than other
vertebrate taxa. We find compelling evidence that cleaner wrasse pass through all stages of
392 the mark test, ultimately attempting to remove the mark when it is able to be viewed in the
mirror (Figure 3). We further find the parsimonious conclusion to be that the behaviours
394 displayed by this fish are equivalent to behaviours taken as evidence for self-recognition in
other taxa (contingency testing, self-directed behaviour, observation and exploration of the
396 body in a reflected image, and removal attempts; Figures 1,2,3). We consider these
behavioural responses to be a consequence of the particular feeding ecology, generally high
398 cognitive capacity, and problem-solving skills of the cleaner wrasse [14-16,37,38]. This is the
first report of successful passing of the mark test in vertebrates outside of mammals and
400 birds, suggesting that if mirror tests are applied considering the cognitive capacities and
ecology of focal species outside of primates, they too may pass the test. Our study further
402 supports previous theories postulating that recognition and cognitive capacities are more
closely related to social and behavioural ecology than relative brain size or phylogenetic
404 proximity to humans [14,16,32].

406 The results we present here will by their nature lead to controversy and dispute, and we
welcome this discussion. We consider three possible interpretations of our results and their
408 significance for understanding the mark test: i) the behaviours we document are not self-
directed and so the cleaner wrasse does not pass the mark test, ii) cleaner wrasse pass the
410 mark test and are therefore self-aware, or iii) cleaner wrasse do pass the mark test but this
does not mean they are self-aware. If one takes position i), rejecting the interpretation that
412 these behaviours are self-directed, it is necessary to demonstrate grounds for this rejection.
As noted above, touching or scraping behaviour is taken as evidence of a self-directed
414 behaviour in mammals, and so if these behaviours are not similarly considered self-directed
in fish, the question must be asked why. For a test to be applicable across species, an
416 objective standard is required. Without such a standard, behaviours assessed in the mark
test can be differently assessed depending on the taxon being investigated. This introduces
418 an impossible, and unscientific, standard for comparison and we therefore reject this
conclusion or must reject the validity of the mark-test entirely.

420

We therefore consider the most parsimonious conclusion to be that the behaviours we
422 observe here in cleaner wrasse are equivalent to those in other taxa during the mirror test.
Based on this, and on the original interpretation of the mark test by its inventor Gallup, who
424 suggested species that pass the mark test are self-conscious and have a true theory of mind
[1,57], would therefore lead us to take position (ii), that cleaner wrasse are self-aware.
426 However, we are more reserved about the interpretation of these behaviours during the mark
test with respect to self-awareness in animals. We do not consider that the successful
428 behavioural responses to all phases of the mark test should be taken as evidence of self-
awareness in the cleaner wrasse, but rather that these fish come to understand that the
430 mirror reflection represents their own body. From the behaviour we observe, we consider the
interpretation that makes fewest assumptions to be that these fish undergo a process of self-
432 referencing, whereby the fish use the mirror to see their own body, but without this involving

theory of mind or self-awareness [32]. This interpretation is supported by a supplementary
434 experiment (Supplementary Figure S1) that showed fish marked on the body in places they
could directly see also performed scraping on those regions.

436

If we therefore accept position (iii), that cleaner wrasse show behavioural responses that fulfil
438 the criteria of the mark test, but that this result does not mean they are self-aware, a question
naturally arises. Can passing the mark-test be taken as evidence of self-awareness in one
440 taxon but not another? A position that holds the same results can be interpreted different
ways depending on where they are gathered is logically untenable, and so must be rejected.
442 This leaves us with the only option to re-evaluate our interpretation of what the mark-test
means, in particular to reject the position that successfully passing the mark test means
444 animals are self-aware and accept that successful performance in the mark test may be
driven by numerous processes. To hold any other dualistic interpretation of the test would be
446 taxonomically chauvinistic and would undermine the standing of the mark test as a valid
metric of self-cognizance in animals (34). Based on our findings, we therefore advocate for a
448 reappraisal of the interpretation of the mark test, and conclude that many more species may
be able to pass the test when it is applied in a manner that is sympathetic to their natural
450 biology.

452 MATERIAL AND METHODS

454 **Animals and housing.** The cleaner wrasse, *Labroides dimidiatus*, is a protogynous
hermaphrodite that lives in coral reef habitats [46,58]. We used 10 wild fish obtained from
456 commercial collectors in this study. Prior to our experiments, the fish were housed in
separate tanks (45 × 30 × 28 cm³, Fig. 1A) and each fish was kept for at least 1 month prior
458 to beginning the experiments to ensure acclimation to captivity and the testing conditions,
and that they were eating and behaving normally. Fish were between 51–68 mm in length;
460 this is smaller than the minimum male size, thus strongly suggesting that these individuals

were functionally female. Individual fish sizes were as follows: 68 mm for fish #1, 62 mm for
462 fishes #13 and #20, 61 mm for fish #21, 58 mm for fish #4, 55 mm for fish #5, 53 mm for fish
#6, 52 mm for fish #2, and #7 and 51 mm for fish #3). Each tank contained a $5 \times 5 \times 10 \text{ cm}^3$
464 rock in the corner, and a PVC pipe that provided shelter on a coral-sand substrate 3–4 cm
deep. The water was maintained at 24–26°C and was aerated and filtered. The fish were
466 maintained on a 12 h: 12 h light: dark cycle. Artificial flake food (Tetramin; Tetra, Blacksburg,
VA, USA) and small pieces of diced fresh shrimp were given twice daily.

468

Mirror presentation to fish. The mirror presentation method (e.g. duration, timing, position
470 and mirror size and shape) has important consequences for successful implementation of
MSR studies [1,4,5]. We presented a $45 \times 30 \text{ cm}^2$ high-quality mirror on a glass wall of the
472 same size inside the experimental tank (Fig. 1A). The mirror was positioned at night, while
the fish were sheltered within the PVC pipe, 1 week before beginning the experiments; it was
474 then completely covered with a white plastic sheet ($45 \times 30 \text{ cm}^2$). At the start of the
experiments, the white cover on the mirror was removed, and the subject fish were exposed
476 to the mirror until the end of the series of experiments, with the exception of a half-day
experiment during which the mirror was completely covered with the white sheet (see below).

478

Order of presentation of the five experiments, E1–E5. We studied fish behavioural
480 responses during five experimental periods, from Experiment 1 (E1) to Experiment 5 (E5) (in
chronological order). All behaviours were recorded by video camera (HDR-CX370; Sony,
482 Tokyo, Japan), and the field of view encompassed the entire experimental tank (Fig. 1A).
After the start of the experiment (i.e. uncovering of the mirror), the behavioural responses of
484 fish were video-recorded eight times over a 2-week period: every day for the first 5 days, and
then again on days 7, 10 and 15 (period E1: initial responses to mirror). Three or four days
486 after the end of period E1, fish behaviours were recorded for 2 consecutive days as a control
for the mark test (E2: no mark). Two or three days after E2, a non-visible sham-mark (white
488 or non-pigmented elastomer) was made on all 10 fish, and behavioural responses were

recorded over the next 2 days (period E3: sham-marking). Two days after period E3, the fish
490 were marked with a coloured (brown) elastomer mark at night, and their behaviours were
recorded in the morning of the following day while the mirror was covered with the 45 × 30
492 cm² white plastic sheet (period E4: colour mark with no mirror present). After this observation
phase, the mirror was uncovered and behaviours were recorded on the afternoon of the
494 same day, and also on the morning of the following day (period E5; colour-mark with mirror
present). All experiments were necessarily run in this order because coloured tags could not
496 be removed from fish once applied; hence, transparent (sham) marks always preceded
coloured marks. The four fish that were marked on the head showed an increase in scraping
498 of the marked area during period E5. However, three of these fish were also observed
scraping facial areas prior to colour marking, indicating that face-scraping cannot be taken as
500 unequivocal evidence of mirror-induced self-directed behaviour.

502 **Provisioning mark procedure.** Elastomer tags and visible implant elastomer (VIE) marking
(Northwest Marine Technology Inc., Shaw Island, USA) via subcutaneous injection are
504 widely used in studies of individually marked live fish and do not affect fish behaviour [51-54,
NMTI]. Our fish were taken from their tanks at night together with their PVC pipe, and placed
506 in eugenol solution to achieve mild anaesthesia (using FA100; Tanabe Pharmacy Inc.,
Japan). An un-pigmented gel mark was injected subcutaneously in an area of 1 × 2 mm² at
508 one of three sites during the sham mark period: on the right side of the head (two fish), on
the left side of the head (two fish) or under the throat (four fish; Fig. 2A). The entire injection
510 process took no longer than 5 minutes, and the fish were returned to their original tank
together with the pipe after the mirror was covered with the white plastic sheet. We ensured
512 that the fish were swimming normally the next early morning, and showed no behavioural
changes as a consequence of the tagging procedure. We initially used white pigment on the
514 pale-coloured body areas, but found that the skin in these areas had a slight blue tint, and
that the white tag was visible in two fish; these fish were not used in further experiments. A
516 brown-pigmented elastomer colour mark was applied as colour-mark at night before the day

of E4. After confirming that all marks were of the same size ($1 \times 2 \text{ mm}^2$), the fish were
518 returned to the tank. Given the location of the tags relative to the field of view of cleaner
wrasse, direct observation of the marks on the head was unlikely, and was definitely
520 impossible for throat marks. To standardise the testing procedure, the brown-coloured mark
was injected at the throat near the transparent marked site. Even with both marks applied,
522 the total volume of the tag was lower than the minimum recommended amount, even for
small fish, and $< 13\%$ of the size of tags used in studies with other fish: biologists who
524 applied VIE to small fish in previous studies, i.e. 26-mm brown trout [51] and 8-mm
damsel fish [54] stated that the amounts used were minute, but for the former species 2–3
526 mm tags were made with 29 G needles [51]. Willis and Babcock used large tags ($10 \times 1 \times 1$
mm (127/ml) in *Pagrus auratus* (from NMTI) [53]. Our own tagging method was therefore
528 very unlikely to have caused irritation. Moreover, we saw no evidence during period E4
(colour tag, no mirror present) of any removal attempts or scratching behaviour, further
530 confirming that the tags did not stimulate the fish.

532 **Behavioural analyses.** Videos of the fish behaviours were used for all behavioural analyses.
Fish performed mouth-to-mouth fighting frequently during period E1, and the duration of this
534 behaviour was recorded (Fig. 1B, Movie S1). Unusual behaviours performed in front of the
mirror, which have never been observed before in a mirror presentation task, nor in the
536 presence of a conspecific, were often observed during the first week of E1, and the type and
frequency of these behaviours was recorded.

538

Description of postural behaviours performed in front of the mirror and behavioural
540 **observations.** In the latter half of E1, fish occasionally swam slowly or remained stationary
in front of the mirror, and the duration (in seconds) of these behaviours, when performed
542 within 5 cm of the mirror, was recorded. The duration of postures in which the marked area
was reflected in the mirror (i.e. viewing behaviours) was recorded during E2 (no mark), E3
544 (sham mark) and E5 (coloured mark with mirror present). Posturing within 5 cm of the mirror

was categorised into three types: right sided posture (i.e. reflecting the right side of the
546 head), left-sided posture (reflecting the left side of the head) and frontal-vertical posture
(reflecting the throat). The duration (in seconds) of each of the three types of posture was
548 recorded during six separate 5-min observation periods, for a total of 30 min per fish for each
of the periods when a mirror was present (E2, E3 and E5). A subset of 15% of the videos
550 was blindly analysed by two researchers outside our team; their analysis was highly
correlated with the main analysis ($r = 0.887$, $P < 0.0001$), and statistical tests showed no
552 significant differences between the two datasets (two-way repeated-measures analysis of
variance [ANOVA], blind effect: $F = 0.06$, $P = 0.80$, blind effect \times observation site: $F = 0.77$, P
554 $= 0.45$).

Scraping behaviour, including the location on the body that was scraped, was observed
556 during periods E2–E5 in the eight subject fish. During period E5, when the fish were colour-
marked and exposed to the mirror, individuals often displayed the marked site to the mirror
558 immediately prior to and following a scraping behaviour. Therefore, we also recorded the
time interval between displaying and scraping during E5.

560

Responses towards real fish. A potential alternative explanation of behaviour in mark tests
562 (and one that is rarely tested for in other vertebrates) is that the focal individual perceives
their reflection not as the self, but rather as another individual behind a glass divide. Although
564 many behaviours seen in the mark test suggest that this is not the case (e.g. contingency-
testing, body exploration), and a growing body of evidence shows that fish perceive mirror
566 reflections in a fundamentally different way to conspecifics behind glass [59,60], we directly
controlled for this possibility by comparing the behaviour of fish confronted with a reflection to
568 that when another individual was across a glass divide.

570 We tested the responses of eight fish (55–59 mm in size) in size-matched pairs. Two fish
were introduced into a tank ($45 \times 30 \times 26$ cm³). After the fish became acclimated, the cover
572 was removed from the divider to allow them to see one another. We then recorded

behavioural responses in the same manner as described for period E1 in the mirror test, for 2
574 weeks. The results of these observation are presented in Fig. 1D. After 3 weeks, we marked
these fish on the throat and recorded whether they scraped their throat regions; however, we
576 did not observe any throat-scraping behaviour, although they must have observed the
'parasite' on the throat of the conspecifics. This indirectly supports the view that the fish were
578 attempting to remove the mark from their own bodies when presented with the mirror during
period E5 of the actual mark test.

580

582 **Statistical analyses.** Statistical analyses were performed using SPSS (ver. 12.0; SPSS Inc.,
Chicago, IL, USA) and R software (ver. 2.13.2; R Development Core Team 2011). During
584 period E1, the responses of the subject fish to the exposed mirror changed significantly over
time. Changes in the duration of mouth fighting and time spent within 5 cm of the mirror over
586 time were analysed with linear mixed models (LMMs). Similarly, changes over time in the
duration of mouth fighting and time spent within 5 cm of the mirror were analysed with LMMs
588 for the experiments using real fish across glass dividers. The frequency of unusual mirror-
testing behaviours was analysed using a generalised linear mixed model (GLMM) with a log-
590 link function and assuming a Poisson distribution. Time spent in postures reflecting the right
side of the head, left side of the head and the throat were compared between mark types
592 during the mark tests (E2: no mark, E3: sham mark and E5: coloured mark with mirror
present) using repeated-measures ANOVA. Note that the marked and unmarked positions
594 were analysed separately (Fig. 2Ca, b). Individual-level statistics on postures that reflected
the marked sites are shown in Table 3 (Mann–Whitney U test with duration in seconds of the
596 six different behaviours per 5-min observation in periods E2, E3 and E5). To detect the effect
of throat marking on the frequency of scraping behaviour, a Friedman test was used on the
598 entire data set (E5 vs. E2, E3 and E4) and a binomial test was used for comparison between
periods (E5 vs. E2, E3 and E4). No throat scraping or unusual behaviours were observed

600 when individuals interacted with conspecifics across a glass divider, so no statistical tests
601 were performed for that condition.

602

Ethics statements. Our experiments were conducted in compliance with the animal welfare
604 guidelines of the Japan Ethological Society, and were specifically approved by the Animal
Care and Use Committee of Osaka City University.

606

SUPPORTING INFORMATION

608

Responses to color dot on flank directly visible and color dot on the mirror. If cleaner
610 fish pass the mark-test, they will also scrape the mark when they detect directly it on its flank
without the aid of mirror. We examined whether cleaner wrasse respond to the mark dot on
612 the flank without mirror. Five other fish of the same size range were kept in experiment tanks
without mirror, and their behaviours were video-recorded for 3 hours in each three conditions
614 in the chronological order of, i) no marking as control, ii) transparent sham marking on the
center of left body side, directly visible for the fish, and iii) colored marking on the left area of
616 the same side of flank, using the same procedure of the mark test. The results are shown in
Supplementary Figure S1. Both sides of the body were scraped on substrate infrequently in
618 the control i) and sham mark ii). In contrast, in the marked phase iii), the marked site in left
flank were frequently rubbed more than right side in the same phase, and than left side of
620 control and sham marking phases (Interaction: $\chi^2 = 12.35$, $df = 2$, $n = 5$, $P < 0.002$), strongly
indicating that cleaner fish regard the directly visible color dot on body surface as
622 ectoparasite.

624 Cleaner wrasse will regard the color dots on body skin as ectoparasite, but do they also
regard the same dots on elsewhere, e.g. on mirror as the ectoparasite? We video-recorded
626 and examined their responses of the other 4 fish to the color dot on mirror on the first day
and 10 days after mirror presentation. On 10 days fish will do MSR. The video-record of 30

628 min showed all fish ignored, hardly watched and approached the color dots on the mirror,
and never stayed in front of it in both days, indicating fish did not pay attention to the dot.

630 Cleaner fish show directed removal attempts toward the mark (Supplementary Figure S1),
indicating that marks on the body are perceived differently to those elsewhere in the

632 environment, whether these bodily marks are observed directly or with a mirror.

634 **Visual and tactile stimuli by the colour mark.** We further considered whether the
elastomer tag could provide a tactile stimulus that, when paired with visual information, may
636 lead to individuals passing the mark test [8,55]. We can effectively rule out that tactile
stimulation alone was sufficient to induce a self-directed behaviour [55] because we
638 observed no throat scraping in any fish during periods E3 (sham mark) and E4 (coloured
mark with no mirror present). This is in contrast to previous studies on rhesus monkeys using
640 a somatosensory training stimulation in which the stimulus immediately elicited a response
due to irritation [10,11] – we observed no such spontaneous response to the tactile stimulus.
642 Moreover, and likely due to the inherent tendency of the species to search for ectoparasites
on the body surface of clients and attempt to remove them [37,45], we did not observe a
644 temporal progression in behaviour that may suggest direct or indirect association learning
[8,10,56], rather a rapid onset of removal attempts in period E5. We therefore conclude that
646 the behaviours we observe were primarily driven by visual stimulation, and required no
association learning, but acknowledge the provision of elastomer tags may provide more
648 tactile stimulation than paint marks [e.g. 1,4]. Future studies should attempt to experimentally
disentangle these two stimuli to assess the roles of visual and tactile stimulation in mirror test
650 with the cleaner wrasse.

652

Acknowledgements

654

We are grateful to numerous conference attendees and anonymous reviewers for their
656 constructive and vigorous advice on the experiments and the manuscript, and to members of
the Laboratory of Animal Sociology, Osaka City University for their support and fruitful
658 discussions. Financial support by a Grant-in-Aid for Scientific Research from the Ministry of
Education, Culture, Sports, Science and Technology (MEXT), Japan (to MK), and by Japan
660 Society for the Promotion of Science (to LAJ) is gratefully acknowledged.

662 **Author contributions.** MK, TT and LAJ conceived and designed the experiments. MK, TT,
TH, HT and JA performed the experiments. SA and HT analysed the data, MK and LAJ wrote
664 the manuscript.

Competing interests. The authors declare no competing interests.

666

668 **SUPPLEMENTARY MATERIALS**

One figure and six movies of captured cleaner wrasse behaviours during the mirror tests, i.e.
670 aggressive fighting, idiosyncratic mirror-testing behaviour, and scraping of coloured marks on
the throat.

672

Supplementary Figure

674

Supplementary Figure S1. The frequencies of rubbing body sides by cleaner wrasse before
676 marking (Control), after transparent marking (Sham) and colour marking (Mark) during 3
hours without mirror. Sham and colour marking were on left flank, the area directly visible for
678 fish ($\chi^2 = 12.35$, $df = 2$, $n = 5$, $P < 0.002$)

680 **Supplementary Movies**

682 **Movie S1.** Mouth-to-mouth fighting against the mirror reflection on the second day after the
initial mirror presentation in phase (*i*) (fish #1). The fish attack the reflection with open
684 mouths during fighting in a common display of fish aggression.

686 **Movie S2.** An example of idiosyncratic behaviour. Mirror-testing behaviour (upside-down
approach) performed by fish #1 on day 4. The fish approached the reflection in an upside-
688 down position, but returned in front of the mirror and viewed the mirror following the
behaviour.

690

Movie S3. An example of idiosyncratic behaviour. Mirror-testing behaviour (dashing along
692 mirror; 'rapid dash') performed by fish #21 on day 4. The fish did not attack the mirror
reflection, but looked at the mirror following the behaviour.

694

Movie S4. Scraping of the throat mark on the sandy bottom by fish #1 immediately after
696 viewing the mark in the mirror. The fish assumed a position that reflects the throat in the
mirror soon after scraping.

698

Movie S5. Fish #1 tried to scrape a throat mark on the sandy bottom immediately after
700 viewing the mark in the mirror, but did not look at its throat in the mirror after scraping.
However, the fish failed to scrape its throat on the sandy bottom, although the sand moved
702 as the fish shook its head. The fish may not have checked its throat in the mirror, possibly
because it had not been scraped.

704

Movie S6. The fish rapidly approached the mirror after scraping its throat on the sandy
706 substrate, stopped at a distance of about 1 cm from the mirror, and remained stationary for 1
s; during this time the fish assumed a position that reflected the scraped throat in the mirror.

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848

Table 1: Description of five types of atypical (mirror- or contingency-testing) behaviours

850 frequently observed during days 3–5 after presentation of the mirror.

(a) Dashing along the mirror: rapid dashing along the mirror surface in a single direction

852 for 10–30 cm. Fish did not swim directly against or make contact with their mirror reflection.

(b) Dashing along the mirror with the head in contact with the mirror: the head of the fish

854 was always in contact with the mirror during dashing.

(c) Dashing and stopping: fish rapidly dashed towards the mirror reflection but stopped

856 before contact with the mirror.

(d) Upside-down approach: fish swam in an upside-down posture while approaching the

858 mirror.

(e) Quick dance: fish spread all of their fins, and quickly arched and quivered the body

860 several times during ca. 1 s at a distance 5–10 cm from the mirror; no dashing to the mirror was observed.

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876 Table 2. Number of atypical (mirror-testing) behaviours shown by seven fish during the 20-
 min observation period in the first 5 days after presenting the mirror. See Table 1 for
 878 description of the behaviours. (a): Dashing along the mirror, (b): Dashing with head in contact
 with the mirror, (c): Dashing and stopping, (d): Upside-down approach, (e) Quick dance

880	Fish	Categories of mirror-testing behaviour					Total
		(a)	(b)	(c)	(d)	(e)	
882	#1	2	0	4	<u>39</u>	0	45
	#2	0	0	<u>30</u>	0	0	30
884	#3	<u>54</u>	0	0	0	0	54
	#7	15	0	0	0	<u>35</u>	50
886	#13	<u>33</u>	0	0	0	0	33
	#20	<u>31</u>	17	2	0	2	52
888	#21	2	0	2	0	<u>6</u>	10
	Total	137	17	38	39	43	274

890 The most frequent mirror-testing behaviour of each fish is underlined.

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896 Table 3. Comparison of time spent in postures in which the marked site was reflected
 between experimental periods of the mark test. Statistical tests (Mann–Whitney *U* test) at the
 898 level of the individual between E2 (no mark) and E3 (sham-mark), between E2 and E5
 (coloured mark) and between E3 and E5. *P*-values, with the *U*-statistic in parentheses, are
 900 shown.

Fish	E2 vs. E3	E2 vs. E5	E3 vs. E5
902	Throat mark		
	#1	<u>0.04</u> * (5.5)	<u>0.004</u> (0.0)
904	#4	0.27 (9.0)	<u>0.004</u> (0.0)
	#20	0.47 (13.5)	<u>0.020</u> (4.0)
906	#21	0.08 (7.0)	<u>0.043</u> (5.5)
	Right-sided head mark		
908	#2	0.31 (9.5)	0.15 (14.5)
	#7	<u>0.01</u> * (0.0)	<u>0.004</u> (0.0)
910	Left-sided head mark		
	#5	0.71 (13.0)	<u>0.004</u> (1.0)
912	#6	0.31 (9.0)	<u>0.011</u> (4.0)

P-values < 0.05 are underlined.

914 * Time in E2 > time in E3 in fish #1, but E2 < E3 in fish #7.

916 **Figure Legends**

918 **Fig. 1. Responses of cleaner wrasse to the mirror and to real fish during the 2-week**
919 **period after the mirror was introduced into the experimental tank.** (A) Design of the
920 experimental tank showing the mirror location. (B) Photograph of mouth-to-mouth fighting
921 against a mirror reflection. (C) Change in social responses towards the mirror. Mean \pm SE for
922 time spent mouth fighting (red), time spent within 5 cm of the mirror without being aggressive
(blue), and frequency of mirror-testing behaviours/10 min (green). Superscript labels a, b and
924 c denote statistical differences. C1: on the seven fish appearing in Table 2; C2: on the three
fish #4, #5 and #6. Statistical results for daily changes in time spent mouth fighting, LMM, c_7^2
926 = 91.87, $P < 0.0001$; time spent in front of the mirror, LMM, $c_7^2 = 64.63$, $P < 0.0001$, and
changes in the number of mirror-testing behaviours, GLMM, $c_7^2 = 137.08$, $P < 0.0001$. (D)
928 Change in social responses to live conspecific fish over 2 weeks: Statistical results for daily
changes in time spent mouth fighting, LMM, $df = 7$, chi-square = 27.36, $P = 0.0003$, and time
930 spent in front of the mirror, LMM, $df = 7$, chi-square = 9.09, $P = 0.25$; no idiosyncratic
behaviours were observed in any fish in this condition. Symbols are as in (C).

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Fig. 2. Mark locations and time spent in a posture allowing viewing of the marked site.

934 (A) Marked location on the left side of the head (left) and under the throat (right); (B)
Postures performed in front of the mirror: (a) displaying right side to mirror, (b) displaying left
936 side to mirror, (c) vertical posture displaying throat, and (d) frontal-vertical posture. (C)
Duration of each posture performed in the mirror for each individual: fish marked on the right
938 side of the head (yellow, $n = 2$), left side of the head (blue, $n = 2$), and throat (red, $n = 4$)
during periods E2 (control: no mark), E3 (sham-mark) and E5 (coloured mark with mirror).
940 (C-a): Time spent viewing the marked location (i.e. the correct side): Repeated-measures
ANOVA, main effect of sequences: $F = 12.09$, $P = 0.016$, marked position: $F = 19.06$, $P =$
942 0.005 , sequence \times marked position: $F = 0.70$, $P = 0.54$. * < 0.05 , ns = not significant ($n = 8$)
(C-b): Time spent viewing unmarked positions. Repeated-measures ANOVA, sequence: $F =$

944 2.54, $P = 0.12$, marked position: $F = 13.15$, $P = 0.0008$, sequence \times marked position: $F =$
946 0.99, $P = 0.42$. The posture allowing head viewing was performed more frequently than the
vertical posture, because vertical swimming is rare in this species.

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Fig. 3. Throat scraping behaviour (self-directed behaviour) and its pattern of

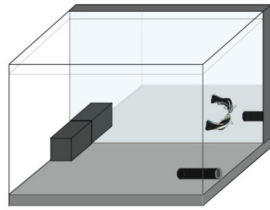
950 **occurrence.** (A) Frame-by-frame example of throat scraping behaviour. (B) Frequency of
throat scraping behaviour of the four throat-marked fish during periods E2, E3, E4 and E5.
952 (C) Schematic sequence of throat scraping behaviour including viewing, and then reviewing,
the throat (before and after scraping, respectively).

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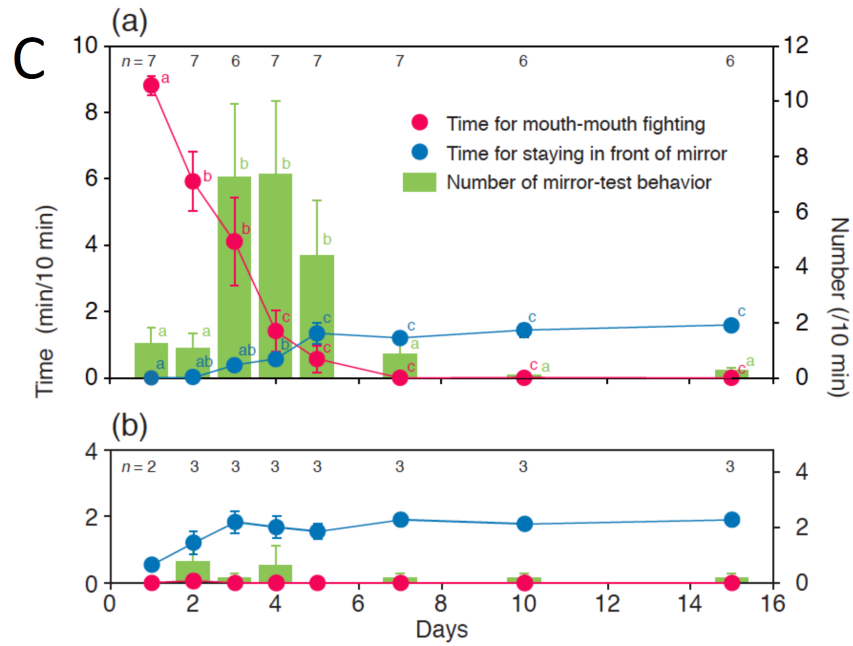
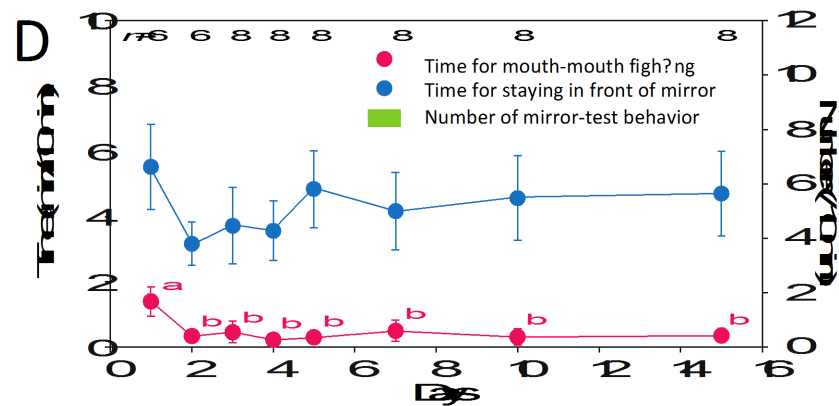
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A

Experiment tank and camera

B

Mouth fight

**D**

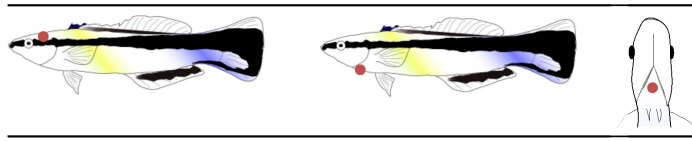
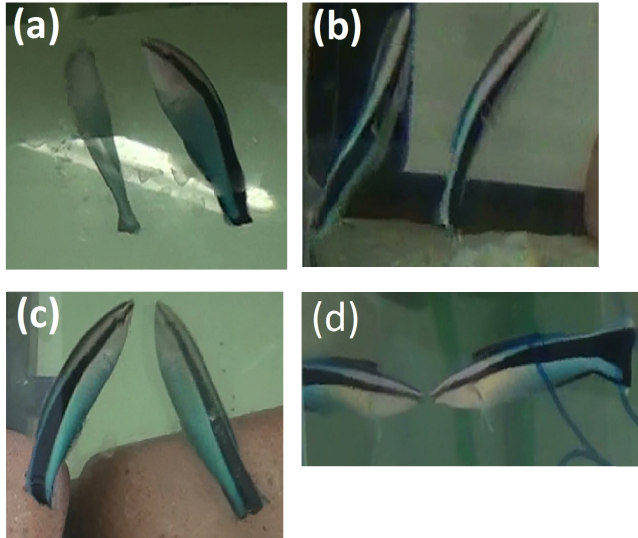
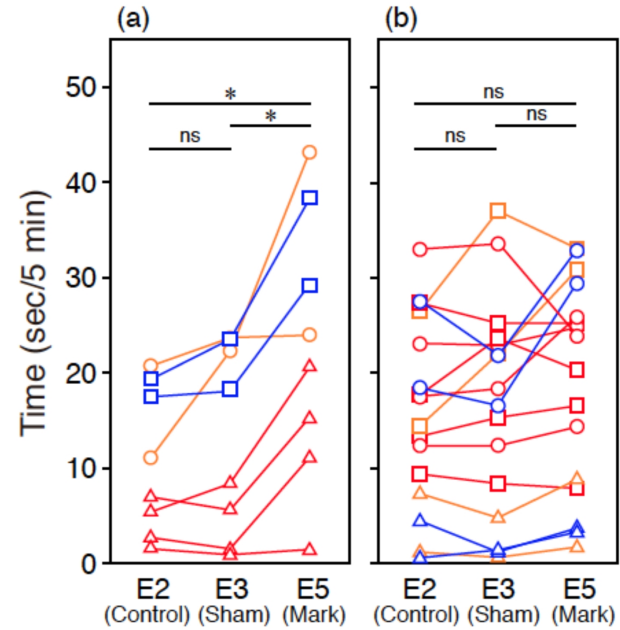
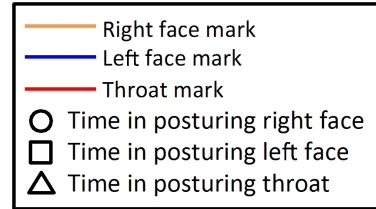
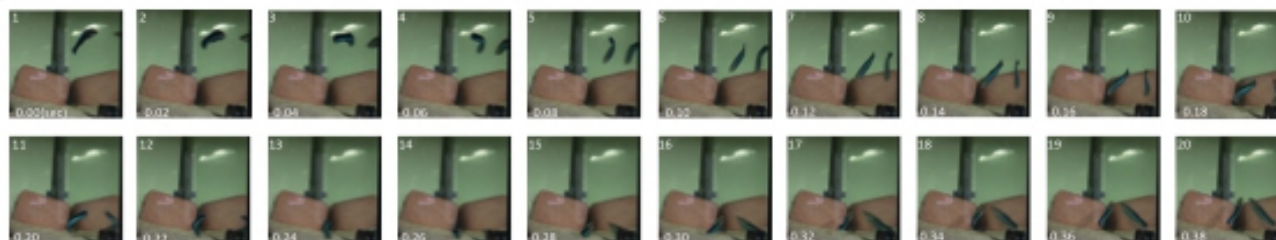
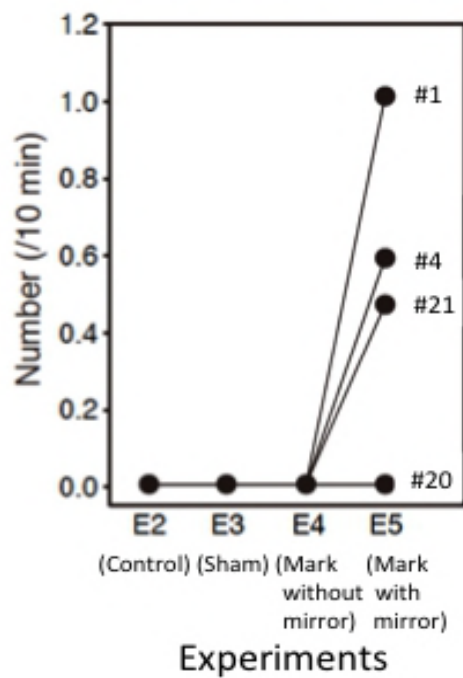
A**B****C**

Figure 2.

A



B



C

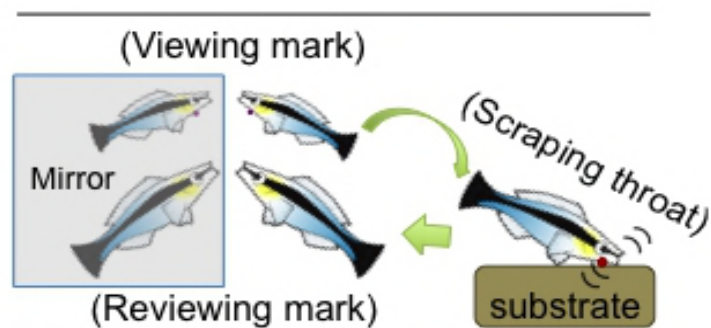


Figure 3.