Applied Animal Behaviour Science 167 (2015) 89–95

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Consistent individual differences in the behavioural responsiveness of adult male cuttlefish (*Sepia officinalis*)

Claudio Carere[a,*], Giacomo Grignani[a], Roberto Bonanni[b], Marco Della Gala[a], Alessandro Carlini[a], Dario Angeletti[a], Roberta Cimmaruta[a], Giuseppe Nascetti[a], Jennifer A. Mather[c]

[a] *Department of Ecological and Biological Sciences, Ichthyogenic Experimental Marine Centre (CISMAR), University of Tuscia, Borgo Le Saline, 01016 Tarquinia, VT, Italy*
[b] *Department of Neuroscience, University of Parma, Italy*
[c] *Department of Psychology, University of Lethbridge, Canada*

ARTICLE INFO

Article history:
Accepted 22 March 2015
Available online 1 April 2015

Keywords:
Cephalopods
Personality
Behavioural consistency
Welfare

ABSTRACT

Consistent individual differences in clusters of behaviour (animal personalities) are being increasingly recognized by researchers of different disciplines, but studies on invertebrates are still scanty. In order to test for the presence of personality-like individual profiles we assessed the behavioural responsiveness of adult male cuttlefish temporarily kept in captivity and individually housed. We used three different stimulations, adapting a protocol established for octopuses (Mather and Anderson, 1993) to assess individual differences (personalities). In the "alerting test" the behaviour was scored after the human observer lifted the cover of the tank. In the "threat test" the behaviour was scored after the animal was touched with a stick. In the "feeding test" the behaviour was scored after the animal was given a live prey. The relationship among behavioural responses was analysed by principal component analysis, which allowed identifying three dimensions along which individual differences were maximal, accounting for 39.6, 15.6, and 10.3% of the total variance. We found marked individual differences that were consistent across the three tests with respect to behaviours such as floating, staying at the bottom of the tank, and moving the fins. However, behaviours such as "moving" and "changes in body patterning" showed context-dependent variation. This study provides evidence of personality dimensions in cuttlefish with implications about welfare of cephalopods used in research in view of the new EU regulations.

1. Introduction

The relevance of studying consistent individual differences in clusters of behaviour (animal personalities) is being gradually and increasingly recognized by researchers of different disciplines (Wolf and Weissing, 2012; Carere and Maestripieri, 2013; Dingemanse and Wolf, 2013). On the applied side, studying and characterizing individual personality is crucial for animal welfare demands, both in animal research and in farming, which require, among other evidence, detailed behavioural assessments at the individual level (Tetley and O'Hara, 2012; Huntingford et al., 2013).

A major challenge in animal personality research is to understand the origin of inter- and intra-individual

* Corresponding author at: Ichthyogenic Experimental Marine Centre (CISMAR), Department of Ecological and Biological Sciences, Università degli Studi della Tuscia, Borgo Le Saline, 01016 Tarquinia, VT, Italy. Tel.: +39 0766 868806.
E-mail address: claudiocarere@unitus.it (C. Carere).

http://dx.doi.org/10.1016/j.applanim.2015.03.005

variation and its universality and maintenance across the animal kingdom, but this effort has been 'vertebrate-centric' so far, being focused especially on birds and mammals. Concomitantly, research on animal welfare has also been almost exclusively focussed on vertebrates. Invertebrates have been traditionally neglected in personality research (Kellert, 1993; Carere et al., 2011; Horvath et al., 2013), with few exceptions (Mather and Anderson, 1993), in spite of the fact they comprise 98% of the animal species on the planet. Only recently a new trend of invertebrate studies has begun (Mather, 2012; Horvath et al., 2013; Kralj-Fiser and Schuett, 2014; Jandt et al., 2014) and research has started to include insects, spiders, crustaceans, cephalopods and other species (e.g. Kralj-Fiser and Schneider, 2012; Gherardi et al., 2012; Mowles et al., 2012). In parallel with these new studies highlighting the presence of personalities as in vertebrates, the attention to invertebrate welfare has also began to increase, given their extensive use for food production and animal experimentation (Horvath et al., 2013; Fiorito et al., 2014; Polese et al., 2014).

Because of their status of 'advanced invertebrates', large behavioural repertoire and flexibility, cephalopods have been evaluated in these areas more thoroughly and for much longer compared to other invertebrates (Hanlon and Messenger, 1996; Zullo and Hochner, 2011; Mather and Kuba, 2013; Fiorito et al., 2014). In octopuses three dimensions were evident in the individual variability: activity, reactivity and avoidance, which accounted for 45% of the total variance in the data (Mather and Anderson, 1993). Sinn and co-workers have conducted in-depth investigation on the small short-lived *Euprymna tasmanica* dumpling squid, including fitness correlates and population differences (Sinn and Moltschaniwskyji, 2005; Sinn et al., 2006, 2008, 2010). Consistency was generally less evident and more context-dependent compared to the octopus studies, including specific temporal windows of major reorganization and stabilization. In general, the personality structure appeared characterized by both continuity and change. Also, evidence for heritability emerged. In a methodologically different study the evidence of consistent individualities was less convincing (Pronk et al., 2010).

Coleoids include three major subgroups, i.e. squid, octopus, and cuttlefish. The third major subgroup of the coleoid cephalopods is the source of much new behavioural investigation, especially in cognitive development (Mather and Kuba, 2013; Darmaillacq et al., 2014a,b), but these organisms have been poorly studied with respect to individuality, and there is just one indication of individual differences in hunting behaviour in *Sepia officinalis* (Adamo et al., 2006). This lack is true despite the fact that cuttlefish have acute vision and extensive learning capacity (Zylinski and Osorios, 2014; Darmaillacq et al., 2014b).

Thus, it is relevant to initiate parallel investigation across the three subgroups. It could be suggested that sociality is a dimension that varies across the three coleoid groups (Boal, 2006) and this could influence the designation of personality dimensions. Although there are obvious differences across the groups, the responses, especially to a potential threat, are often similar. For instance, all three groups have the ability to change colour due to the chromatophore skin display system and use these changes in response to both potential predators and conspecifics (Hanlon and Messenger, 1996; Zylinski and Osorios, 2014). Octopuses and cuttlefish match the appearance of the substrate on which they rest (Hanlon and Messenger, 1988). Cuttlefish (Langridge, 2009) and squid (Mather, 2010) use an eyespot display in response to the approach of predators, and animals from all three groups use jet-propelled escape with release of ink to a major threat. With respect to the degree of social complexity, cuttlefish (Sepioidea) are at an intermediate level between the solitary octopuses (Octopoda) and the schooling squid (Teuthoidea). Once personality comparisons are carried out and differences have been found, we can begin to look at how individual differences affect performance and how variation in the environment might affect them.

Research on individual differences and personality is also prompted by the recent inclusion of "all live cephalopods" in Directive 2010/63/EU on the use of animals for scientific purposes based on "scientific evidence of their ability to experience pain, suffering, distress and lasting harm" (European Parliament and Council of the European Union, 2010). Such an inclusion is not shared by the entire scientific community (e.g. Mason, 2011; Sneddon et al., 2014), as well as by all stakeholders, thus it is instrumental to objectively study new areas that could contribute to its understanding (see Crook et al., 2011 for squid). Personality is one of such aspects, since knowing about individual difference will allow us to tailor the captive environment to the individual, not just to the species (Horvath et al., 2013). Personality assessment falls within non-invasive approaches in characterizing behaviour and physiology, an area suggested to contribute to increasing knowledge of cephalopod welfare (Fiorito et al., 2014). Furthermore, among the approximately 700 extant cephalopod species, those belonging to the coleoid group, including the cuttlefish (*S. officinalis*), are the most commonly used in research (Fiorito et al., 2014).

The goal of this study was to qualitatively and quantitatively describe individual differences in cuttlefish using repeated behavioural measures across three different contexts, together with their degree of consistency. Some evidence of consistent individual differences was previously found in a relatively small sample of cuttlefish with respect to their tendency to use raised arms during hunting (Adamo et al., 2006). For this reason and also due to previous studies in other cephalopods species, we expected to find consistent individuality in our study.

2. Material and methods

2.1. Subjects and housing

Cuttlefish (*S. officinalis*) were captured by nets offshore in the coastal area between Montalto Marina and Tarquinia (Central Tyrrhenian Sea, Italy), at a depth ranging between 2.5 and 7.5 m, and placed in a live well in a boat (80 cm × 40 cm × 30 cm, capacity 90 l). After the return to shore, they were placed into a barrel (36 cm Ø, 36 cm height) for transportation to the laboratory by car, lasting about 30 min. We selected males, as females were mature and their behaviour would be changed if egg laying occurred. Previously, sex did not affect personality in octopuses (Mather and Anderson, 1993). We tested two groups that were fished at different times, comprising twelve individuals and nine individuals, respectively. The first group started tests on 30-3-2011, the second one on 19-4-2011. They were individually housed in glass tanks (60 cm × 30 cm × 35 cm, capacity 60 l) with water that was both mechanically and biologically filtered, exposed to a UV sterilizer and circulated in a closed system at 18 °C, salinity 38 psu, pH 8.2–8.4, ammonia and nitrite absent, nitrate range 20–50 mg/l. The tanks were adjacent to each other in two rows of six, but the animals inside had no visual contact with the surrounding environment as black plastic covered all tank sides and tops. They were fed one live fish (*Mugil cephalus*, length 6–10 cm) per day as part of the study. Illumination was set at a 12:12 light–dark cycle (light on at 8:00 am and off at 8:00 pm). The animal's size was measured as mantle length with callipers to the nearest 0.1 cm just before housing. Mean size (±sd) was 13.52 ± 1.76 cm. Animals were kept 7–10 days for acclimation before starting the tests. This study precedes the implementation of the new regulations in Italy (see Fiorito et al., 2014 for European regulations regarding cephalopod research), but complied with the already implemented regulations of the Canadian Council of Animal Care for animal research (University of Lethbridge, Animal Welfare Approval #1106).

2.2. Testing procedure

Cuttlefish were tested one at a time, all at the same times of day, and all tests were video-recorded (Canon MV890) on mini-DV tapes. Every day each individual received three tests: the first two (alerting and threat) were performed in the morning, but the third (feeding) was separated from them by a delay of at least 3 h (see procedures of Mather and Anderson, 1993). (1) *Alerting* (A): the black plastic lid of the tank was opened and the observer (G.G.) brought his head over the water surface (at a distance of about 25 cm) where the cuttlefish could see it clearly. He looked directly at it for one minute, and then moved out of view. Cuttlefish behaviours were recorded for this minute and five subsequent ones. (2) *Threat* (T): the observer extended a long test-wooden stick (0.5 cm diameter, 80 cm long) into the tank and touched the cuttlefish with its end; contact was maintained for approximately 1 s, and the typical area contacted was midway along the lateral side of the mantle. The stick was then withdrawn from the tank and the cuttlefish behaviour was filmed for the subsequent 5 min. (3) *Feeding* (F): a live fish (*M. cephalus*, 6–10 cm long) was dropped into the side of the tank opposite to the one where the cuttlefish was located. The cuttlefish behaviour was filmed for the next five minutes. If the fish was not eaten it was left there until the next morning. Tests were carried out for 10 days (5 consecutive days, a break of 2 days and 5 more consecutive days). On each day, after the second test (threat) the tank of each animal was cleaned by a siphon.

The behavioural variables considered were: *floating*: individual motionless in the water column (duration, measured in A, T, F); *bottom of the tank*: individual motionless at the bottom (duration, measured in A, T, F); *moving the fins*: fin movement while motionless in any part of the tank (duration, measured in A, T, F); *attacking a prey*: a sequence of attack starting from the moment in which the first two arms are lifted close to each other and directed towards the prey (duration, measured in F); *escape*: jet-propelled rapid escape response (duration, measured in T); *changing colour*: rapid colour change of the mantle skin through the chromatophores (frequency, measured in A, T, F); *eyespot*: emergence of two distinct black spots on the posterior–dorsal mantle (duration, measured in A, T, F); *moving*: individual in locomotory activity (duration, measured in A, T, F). In cephalopods displaying colour changes and eyespots represent the so-called deimatic behaviour considered to be functional in startling a predator (Hanlon and Messenger, 1996; King and Adamo, 2006). Some individuals also displayed ink ejection (during escape reactions), but they were too few to be treated in a statistical analysis. See supplementary material for a representative compilation of the behavioural variables.

2.3. Data collection and statistical analysis

Data were scored by a single operator (G.G.) with the software Observer 2.0 (Noldus). We used Principal Component Analysis (PCA) to describe the relationship between specific behaviours of cuttlefishes measured in the three different tests. This is a multivariate statistical approach (for a detailed description see Giuliani et al., 1994, 2004) that has been employed successfully to investigate personality in several taxa (e.g. Mather and Anderson, 1993; Seaman et al., 2002; Natoli et al., 2005; Ferrari et al., 2013). It allows a reduction of the dimensionality of the dataset by replacing multiple inter-related original variables with a few, new uncorrelated component variables called "factors". Each factor represents a linear combination of the original variables, and it is constructed to have a mean value of zero and a standard deviation of one. The factors are extracted in order of decreasing percentage of variation that they explain of the whole data set, so the highest the variation explained by a given factor, the highest the elongation of the data points along it. Note that, although the scores of each factor are calculated by linearly combining all the original variables, factors differ with respect to the regression coefficients (weights) assigned to the original variables, which in turn are related to the correlation coefficients between the factors themselves and the original variables. An examination of the factor-variable correlations allows us to assess: (1) the differences between factors; (2) which original variables are more influenced by variations in the underlying factor; (3) which original variables covary along the same dimension (e.g. when they show a substantial correlation with the same factor). So, this analysis allowed us: (i) to identify the dimensions along which individual differences in cuttlefish behaviour were maximal (PCA factors); (ii) to assess the individual consistency of the behavioural patterns across the three tests (based on factor-original variable correlation) and consequently (iii) the degree of behavioural plasticity exhibited by the animals (intended as reduced consistency of behavioural patterns across the three tests). We entered in the PCA all the original variables described in the previous section, averaging the ten replicas of each test.

Note that we ran the PCA by pooling the animals of the two groups together ($N = 21$). This was done in order to increase the sample size, and it was justified because the groups did not differ significantly with respect to nearly all of the variables entered. The only exception was "duration of escape behaviour in T", for which the first group scored significantly higher than the second (Mann–Whitney U test: $U = 20$; $Z = 2.42$; $P = 0.016$).

3. Results

In Table 1 we report descriptive statistics concerning the scores recorded in each test for all the original variables entered in the PCA. The first factor of the PCA explained about 39.6% of the total variance in the data, the second about 15.6%, and the third about 10.3%. The first factor was highly and positively correlated with the time spent at the bottom of the tank (in A, T, F), and it was highly and negatively correlated to time spent floating (in A, T, F). Moreover, it showed a negative correlation, ranging from moderate to high (<-0.4) with: "moving the fins" (in A, T, F), total time spent moving (in A, F), "changing colour" in T, and "eyespot" in T (Table 2). In other words, individuals who had high negative scores on the first factor spent considerable time floating in all three tests (and very little time at the bottom of the tank), spent considerable time moving in A and F (although moved less in T), spent considerable time moving the fins (in A, T, F), changed colour frequently in T, and showed eyespot for long durations in T. Conversely, individuals with high positive scores on the first factor spent considerable time at the bottom of the tank in all three tests (and very little time floating), spent little time moving (in A, F), spent little time moving the fins in all three tests, changed colour rarely and did not show eyespot for long durations when exposed to a threat.

The second factor showed a high negative correlation with moving in T, and a moderate, negative correlation with moving in A, with eyespot in T and F, and with changing colour in T. Moreover, it had a good positive correlation with moving the fins in A, T, and F (Table 2). So, animals with high negative scores on the second factor spent considerable time moving especially during the T test, changed colour frequently in T and showed eyespot for long durations (in T, and F). Moreover, they spent little time moving the fins in all three tests. The opposite pattern of covariation of variables held for individuals who had high positive scores on this factor.

The third factor had a high and negative correlation with changing colour in A and F. It also had a negative, moderate correlation with attacking behaviour in F, and a positive moderate correlation with escape in T, as well as with eyespot in A (Table 2). So, individuals with high negative scores on the third factor changed colour frequently especially in A and F, showed attacking behaviour towards a prey for long durations, whereas they showed short durations of escaping behaviour in T, as well as short duration of eyespot in A. The opposite pattern of covariation of variables held for animals having high positive scores on this factor.

Table 1
Measures of behavioural responses of individual cuttlefish ($N = 21$) in tests of alerting, threat and feeding. Values are mean durations ± standard deviation in seconds (except for item with * in which mean frequency of occurrence ± standard deviation is reported). See method section for details on behavioural variables.

Behaviour	Mean ± SD	Coefficient of variation	Test
			Alerting
Bottom	258.45 ± 100.11	38.74	
Floating	100.92 ± 99.78	98.87	
Moving fins	114.46 ± 56.94	49.74	
Changing colour*	10.74 ± 5.76	53.67	
Eyespot	5.63 ± 8.94	158.74	
Moving	52.53 ± 42.54	80.98	
			Threat
Bottom	221.51 ± 79.91	36.07	
Floating	78.20 ± 77.30	98.85	
Moving fins	93.68 ± 60.64	64.73	
Escape	5.42 ± 9.11	168.03	
Changing colour*	9.99 ± 5.67	56.76	
Eyespot	0.57 ± 0.91	158.87	
Moving	32.03 ± 23.93	74.72	
			Feeding
Bottom	231.12 ± 93.89	40.62	
Floating	68.17 ± 93.05	136.49	
Moving fins	139.85 ± 54.12	38.70	
Attack	19.37 ± 16.13	83.28	
Changing colour*	12.50 ± 5.18	41.49	
Eyespot	3.04 ± 4.89	160.88	
Moving	20.37 ± 20.93	102.76	

4. Discussion

This study provides evidence of personality dimensions in *S. officinalis*, a widely used species of cephalopods, i.e. the only invertebrate taxon included in the new EU regulations concerning welfare of animals used in research (Fiorito et al., 2014). The results indicate that all the behavioural variables considered were characterized by a good level of individual consistency, either in all three (for time spent at the bottom, time spent floating, moving fins), or in at least two tests (for moving, changing colour, eyespot), by loading on the same PCA factor with the same sign (Table 2). This suggests that the PCA factors represented underlying dimensions simultaneously affecting multiple behavioural measures (i.e. causing a parallel increase/decrease in the values of such measures) which, in turn, would be indicative of personalities as found in several other invertebrate species, including cephalopods (Mather and Logue, 2013; see Table 3), as well as on a previous study in this species carried out with a smaller sample size (Adamo et al., 2006).

The first factor of the PCA represented the dimension along which individual differences were maximal. Specifically, it showed that levels of floating covaried (with the same sign) with levels of activity, expressed both through moving and moving the fins, and that all these behavioural measures were rather consistent across the tests. Moreover, this factor indicates that active individuals were usually those reacting to a threat by changing their colour and by displaying eyespot. So, we could say that this factor discriminated individuals based on their overall consistency in activity and on their deimatic behaviour in response to a threat. This result is comparable to that found by Mather and Anderson (1993) for octopuses (*Octopus*

Table 2
Pearson's correlations between the first three factors of the PCA and the behavioural variables under investigation across the three tests (A: alerting; F: feeding; T: threat). Moderate to high correlations (>0.4 and <−0.4) are highlighted in bold and were used to interpret the meaning of the PCA factors. Significance values are reported in parentheses.

	Factor 1 (39.6% total variance)	Factor 2 (15.6% total variance)	Factor 3 (10.3% total variance)
A Bottom	**0.957** (0.0001)	−0.051 (0.828)	−0.038 (0.870)
T Bottom	**0.957** (0.0001)	0.077 (0.739)	−0.067 (0.773)
F Bottom	**0.831** (0.0001)	−0.284 (0.211)	−0.100 (0.666)
A Floating	−**0.958** (0.0001)	0.053 (0.819)	0.048 (0.836)
T Floating	−**0.959** (0.0001)	−0.066 (0.775)	0.053 (0.818)
F Floating	−**0.843** (0.0001)	0.283 (0.214)	0.122 (0.599)
A Moving fins	−**0.621** (0.003)	**0.549** (0.010)	0.166 (0.473)
T Moving fins	−**0.543** (0.011)	**0.523** (0.015)	0.214 (0.351)
F Moving fins	−**0.451** (0.040)	**0.681** (0.001)	−0.018 (0.940)
F Attack	0.143 (0.536)	0.365 (0.103)	−**0.402** (0.071)
T Escape	0.307 (0.176)	−0.101 (0.665)	**0.443** (0.044)
A Change colour	−0.173 (0.454)	−0.041 (0.861)	−**0.770** (0.0001)
T Change colour	−**0.606** (0.004)	−**0.455** (0.038)	−0.324 (0.152)
F Change colour	−0.379 (0.090)	−0.065 (0.781)	−**0.741** (0.0001)
A Eyespot	0.240 (0.294)	−0.268 (0.241)	**0.528** (0.014)
T Eyespot	−**0.431** (0.051)	−**0.611** (0.003)	−0.057 (0.805)
F Eyespot	−0.166 (0.472)	−**0.493** (0.023)	−0.001 (0.997)
A Moving	−**0.628** (0.002)	−**0.439** (0.046)	0.120 (0.604)
T Moving	−0.326 (0.150)	−**0.743** (0.0001)	0.085 (0.714)
F Moving	−**0.738** (0.0001)	−0.326 (0.149)	0.193 (0.403)

rubescens), in which the first factor of the PCA also separated individuals based on their level of activity. However, in that study, the first factor of the PCA explained a smaller portion of the total variance in the data.

The interpretation of the other two factors of the PCA, which explained a smaller proportion of the overall variation present in the data, appears more difficult. High levels of both mobility and deimatic reactivity during the threat test characterized the second factor. One interpretation is that this factor discriminated animals based on their "conditional activity/reactivity in response to a threat". However, for some reason, an increase in activity, as well as in (deimatic) reactivity, during the threat test was inversely related to the frequency of moving the fins. This factor may represent reactivity as in octopuses (Mather and Anderson, 1993).

The third factor was characterized by high frequencies of changing colour during both the feeding and alert tests that covaried with prolonged attacking behaviour towards a prey during the feeding test. So, On the one hand, this factor may be regarded as a measure of "activity/reactivity during feeding". On the other hand it was the only factor having a statistically significant correlation with duration of escape behaviour from a threat (Table 2). So, it may also represent an avoidance axis as in octopuses (Mather and Anderson, 1993), often described as a shy-bold axis by researchers on vertebrate personality (e.g. Natoli et al., 2005; Ferrari et al., 2013). "Duration of escape behaviour" was the only original variable for which we detected a statistically significant difference between the two (pooled) groups of captured animals. However, this variable had a meaningful correlation only with a factor explaining a small portion of the overall variance in the data (Table 2), and repeating the analysis after excluding it did not result in any substantial change of the overall results of the PCA.

Such results are in line with those found in other cephalopod species studied with a "bottom-up approach", i.e. testing animals in common situations and analysing

Table 3
Cephalopod studies assessing consistent behavioural differences over time, situations and/or contexts. Among tests: correlations conducted between variables measured in different tests; BS tested: correlations between behaviours tested (behavioural syndrome). Modified from Kralj-Fiser and Schuett (2014).

Species	Common name	Behavioural trait(s)	Time consistency tested	Situation consistency tested	Evidence time/situation consistency	Context consistency/BS tested	Study
Octopus rubescens	Red octopus	Threat response, feeding	Yes, short term	No	Partly	Yes (among tests)	Mather and Anderson (1993)
Euprymna tasmanica	Dumpling squid	Threat response, feeding	Yes	No	Yes	Yes (among tests)	Sinn and Moltschaniwskyji (2005)
Euprymna tasmanica	Dumpling squid	Threat response, feeding	Yes	Yes,	Yes	Yes (among tests)	Sinn et al. (2008)
Octopus tetricus	Gloomy octopus	Response to conspecific video	Yes	No	Episodic	Yes (among tests)	Pronk et al. (2010)
Sepia officinalis	Cuttlefish	Threat response, feeding	Yes, short term	No	Partly	Yes (among tests)	This study

behaviour with multivariate statistical methods without a priori expectations in order to detect patterns of covariation of variables that may correspond to personality axes (Table 3). The basic finding of these studies, carried out in *O. rubescens* (Mather and Anderson, 1993), in young *O. bimaculoides* (Sinn et al., 2001), and in *E. tasmanica* (Sinn and Moltschaniwskyji, 2005) was that cephalopods exhibit a set of personality axes similar to those found in vertebrates, with three or four axes accounting for a range from 44.9% to 78.4% of the total variance (Mather and Logue, 2013; see Gosling, 2001 for a comparison with vertebrates).

Our results suggest that, among coleoids, cuttlefish are not an exception to the pattern of animals showing consistent individual differences in behaviour and this has a number of implications in relation to the main points put forward in the introduction. First of all, they suggest the presence of individual personality profiles, which strengthens the recent emphasis to broaden the comparative animal personality research to invertebrates in order to advance understanding of crucial questions in the field (Kralj-Fiser and Schuett, 2014). In this respect, cuttlefish are useful animals especially to study ontogeny of personality and possibly early experiences and maternal effects, which are critical and understudied issues (Groothuis and Trillmich, 2011; Darmaillacq et al., 2014a,b). Cephalopods are also attractive for studies on the emergence of sex differences in personality, because sexual maturation occurs relatively late in life (Mather, 2006). Second, our results support the view that cephalopods indeed deserve ethical consideration, as prompted by the new EU regulation. They support tailoring their care so that conditions fit the individual, not the whole species (e.g. a shy animal might need more protection from onlookers, an active one less opportunity to escape). This study also suggests that, despite their position on the sociality dimension (Boal, 2006), cephalopods of the three orders appear to have similar ranges and categories of personality. The evidence of individual differences comparable to personalities adds to other recent discoveries on the abilities of these animals, such as tactical intra-specific deception (Brown et al., 2012). Guidelines for behaviour and welfare assessment should carefully consider the presence of individualities in cuttlefish, not only in the framework of animal experimentation, but also with respect to its potential for aquaculture (Sykes et al., 2006).

Acknowledgments

This research was supported by a University of Lethbridge Research Grant to J.A. Mather. We thank Riccardo Delle Fratte and Rossana Giannarini for practical and administrative support, and Alessandro Giuliani for his valuable statistical advice. The work is part of the BSc thesis of G. Grignani.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.applanim.2015.03.005.

References

Boal, J.G., 2006. Social recognition: a top-down view of cephalopod behaviour. Vie Mil 56, 69–79.

Adamo, S.A., Ehgoetz, K., Sangster, C., Whitehorne, I., 2006. Signaling to the enemy? Body pattern expression and its response to external cues during hunting in the cuttlefish *Sepia officinalis* (Cephalopoda). Biol. Bull. 210, 192–200.

Brown, C., Garwood, M.P., Williamson, J.E., 2012. It pays to cheat: tactical deception in a cephalopod social signaling system. Biol. Lett. 8, 729–732.

Carere, C., Maestripieri, D. (Eds.), 2013. Animal Personalities: Behavior, Physiology, and Evolution. The University of Chicago Press, Chicago.

Carere, C., Wood, J.B., Mather, J.A., 2011. Species differences in captivity: where are the invertebrates? Trends Ecol. Evol. 26, 211.

Crook, R.J., Lewis, T., Hanlon, R.T., Walters, E.T., 2011. Peripheral injury induces long-term sensitization of defensive responses to visual and tactile stimuli in the squid Loligo pealeii Lesueur 1821. J. Exp. Biol. 214, 3173–3185.

Darmaillacq, A.-S., Dickel, L., Mather, J.M. (Eds.), 2014a. Cephalopod Cognition. Cambridge University Press, Cambridge, pp. 3–23.

Darmaillacq, A.-S., Dickel, L., Jozet-Alves, C., Bellanger, C., 2014b. Cuttlefish preschool or how to learn in the perihatching period. In: Darmaillacq, A.-S., Dickel, L., Mather, J.M. (Eds.), Cephalopod Cognition. Cambridge University Press, Cambridge.

Dingemanse, N.J., Wolf, M., 2013. Between-individual differences in behavioural plasticity within populations: causes and consequences. Anim. Behav. 85, 1031–1039.

Ferrari, C., Pasquaretta, C., Carere, C., Cavallone, E., von Hardenberg, A., Reale, D., 2013. Testing for the presence of coping style in a wild mammal. Anim. Behav. 85, 1385–1396.

Fiorito, G., Affusso, A., Anderson, D.B., Basil, J., Bonnaud, L., Botta, G., Cole, A., et al., 2014. Cephalopods in neuroscience: regulations research, and the 3Rs. Invert. Neurosci. 14, 13–36.

Gherardi, F., Aquiloni, L., Tricarico, E., 2012. Behavioural plasticity, behavioural syndromes and animal personality in crustacean decapods. An imperfect map is better than no map. Curr. Zool. 58, 567–579.

Giuliani, A., Ghirardi, O., Caprioli, A., Di Serio, S., Ramacci, M.T., Angelucci, L., 1994. Multivariate analysis of behavioural aging highlights some unexpected features of complex systems organization. Behav. Neural Biol. 61, 110–122.

Giuliani, A., Zbilut, J.P., Conti, F., Manetti, C., Miccheli, A., 2004. Invariant features of metabolic networks: a data analysis application on scaling properties of biochemical pathways. Physica A 337, 157–170.

Gosling, S.D., 2001. From mice to men: what can we learn about personality from animal research? Psychol. Bull. 127, 45–86.

Groothuis, T.G.G., Trillmich, F., 2011. Unfolding personality: the importance to study ontogeny. Dev. Psychobiol. 53, 641–655.

Hanlon, R.T., Messenger, J.B., 1988. Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behavior. Phil. Trans. R. Soc. Lond. B 320, 437–487.

Hanlon, R.T., Messenger, J.B., 1996. Cephalopod Behaviour. Cambridge University Press, Cambridge.

Horvath, K., Angeletti, D., Nascetti, G., Carere, C., 2013. Invertebrate welfare: an overlooked issue. Ann. Ist. Super. Sanita 49, 9–17.

Huntingford, F., Mesquita, F., Kadri, S., 2013. Personality variation in cultured fish: implications for production and welfare. In: Carere, C., Maestripieri, D. (Eds.), Animal Personality, Behavior, Physiology, and Evolution. University of Chicago Press, Chicago.

Jandt, J.M., Bengston, S., Pinter-Wollman, N., Pruitt, J.N., Raine, N.E., Dornhaus, A., Sih, A., 2014. Behavioral syndromes and social insects: personality at multiple levels. Biol. Rev. Camb. Philos. Soc. 89, 48–67.

Kellert, S.R., 1993. Values and perceptions of invertebrates. Cons. Biol. 7, 845–855.

King, A.J., Adamo, S.A., 2006. The ventilatory cardiac and behavioural responses of resting cuttlefish (*Sepia officinalis* L.) to sudden visual stimuli. J. Exp. Biol. 208, 1101–1111.

Kralj-Fiser, S., Schneider, J.M., 2012. Individual behavioural consistency and plasticity in an urban spider. Anim. Behav. 84, 197–204.

Kralj-Fiser, S., Schuett, W., 2014. Personality variation in invertebrates: why bother? Anim. Behav. 91, 41–52.

Langridge, K.V., 2009. Cuttlefish use startle displays but not against large predators. Anim. Behav. 77, 847–856.

Mason, G.J., 2011. Invertebrate welfare: where is the real evidence for conscious affective states? Trends Ecol. Evol. 26, 212–213.

Mather, J.A., Anderson, R.C., 1993. Personalities of octopuses (*Octopus rubescens*). J. Comp. Psychol. 107, 336–340.

Mather, J.A., 2006. Behaviour development: a cephalopod perspective. Int. J. Comp. Psychol. 19, 98–115.

Mather, J.A., 2010. Vigilance and antipredator responses of Caribbean reef squid. Mar. Freshw. Behav. Physiol. 43, 357–370.

Mather, J.A., 2012. Why (and how) personalities in invertebrates? Curr. Zool. 58, 566–570.

Mather, J.A., Kuba, M.J., 2013. The cephalopod specialties: complex nervous system learning, and cognition. Can. J. Zool. 91, 431–449.

Mather, J.A., Logue, D.M., 2013. The bold and the spineless: approaches to personality in invertebrates. In: Carere, C., Maestripieri, D. (Eds.), Animal personalities: Behavior, Physiology and Evolution. University of Chicago Press, Chicago.

Mowles, S.L., Cotton, P.A., Briffa, M., 2012. Consistent crustaceans: the identification of stable behavioral syndromes in hermit crabs. Behav. Ecol. Sociobiol. 66, 1087–1094.

Natoli, E., Say, L., Cafazzo, S., Bonanni, R., Schmid, M., Pointier, D., 2005. Bold attitude makes urban feral domestic cats more vulnerable to feline immunodeficiency virus. Neurosci. Biobehav. Rev. 29, 151–157.

Polese, P., Winlow, W., Di Cosmo, A., 2014. Dose-dependent effects of the clinical anesthetic isoflurane on *Octopus vulgaris*: a contribution to cephalopod welfare. J. Aquat. Anim. Health 26, 285–294.

Pronk, R., Wilson, D.R., Harcourt, R., 2010. Video playback demonstrates episodic personality in the gloomy octopus. J. Exp. Biol. 213, 1035–1041.

Seaman, S.C., Davidson, H.P.B., Waran, N.K., 2002. How reliable is temperament assessment in the domestic horse (*Equus caballus*)? Appl. Anim. Behav. Sci. 78, 175–191.

Sinn, D.L., Perrin, N., Mather, J.A., Anderson, R.C., 2001. Early temperamental traits in an octopus (*Octopus bimaculoides*). J. Comp. Psychol. 115, 351–364.

Sinn, D.L., Moltschaniwskyji, N.A., 2005. Personality traits in dumpling squid (*Euprymna tasmanica*): context-specific traits and their correlation with biological characteristics. J. Comp. Psychol. 119, 99–110.

Sinn, D.L., Apiolaza, L.A., Moltschaniwskyj, N.A., 2006. Heritability and fitness-related consequences of squid personality traits. J. Evol. Biol. 19, 1437–1447.

Sinn, D.L., Gosling, S.D., Moltschaniwskyj, N.A., 2008. Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. Anim. Behav. 75, 433–442.

Sinn, D.L., Moltschaniwskyj, N.A., Wapstra, E., Dall, S.R.X., 2010. Are behavioral syndromes invariant? Spatiotemporal variation in shy/bold behaviour in squid. Behav. Ecol. Sociobiol. 64, 693–702.

Sneddon, L.U., Elwood, R.W., Adamo, S.A., Leach, M.C., 2014. Defining and assessing animal pain. Anim. Behav. 97, 201–212.

Sykes, A.V., Domingues, P.M., Correia, M., Andrade, J.P., 2006. Cuttlefish culture – state of the art and future trends. Vie Milieu A: Biol. Ma 56, 129–137.

Tetley, C.L., O'Hara, S.J., 2012. Rating of personality as a tool for improving the breeding management and welfare of zoo animals. Anim. Welf. 21, 463–476.

The European Parliament and the Council of the European Union Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes., 2010. Official J. Eur. Union 53, 33–79.

Wolf, M., Weissing, F.J., 2012. Animal personalities: consequences for ecology and evolution. Trends Ecol. Evol. 27, 452–461.

Zylinski, S., Osorios, D., 2014. Cuttlefish camouflage: vision and cognition. In: Darmaillacq, A.-S., Dickel, L., Mather, J.M. (Eds.), Cephalopod Cognition. Cambridge University Press, Cambridge, pp. 197–217.

Zullo, L., Hochner, B., 2011. A new perspective on the organization of an invertebrate brain. Commun. Integr. Biol. 4, 26–29.