



基于眼睛晶体核心微量元素的茎柔鱼地理种群判别

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摘要: 实验以2015年和2017年我国远洋鱿钓渔船在东南太平洋的厄瓜多尔、秘鲁和智利专属经济区外海生产调查时所采集的茎柔鱼眼睛晶体为研究材料, 采用激光剥蚀—电感耦合等离子体质谱法(laser ablation inductively coupled plasma mass spectrometry, LA-ICP-MS)测定了眼睛晶体核心的微量元素, 并采用逐步判别分析(stepwise discriminant analysis, SDA)法研究了基于微量元素的茎柔鱼地理种群划分。结果显示, 厄瓜多尔、秘鲁和智利外海三海区之间的茎柔鱼眼睛晶体核心微量元素含量差异显著(除Al²⁷和Si²⁹以外)。眼睛晶体核心的微量元素可以用来判别茎柔鱼不同地理种群, 总体判别成功率为69.2%, 厄瓜多尔、秘鲁和智利外海的判别成功率分别为87.0%、70.0%和52.0%。若将秘鲁与智利外海两海区样本合并后再与厄瓜多尔进行判别, 则总体判别成功率上升至89.7%, 厄瓜多尔、秘鲁与智利外海分别为95.70%和87.30%。Mg²⁵可将厄瓜多尔与秘鲁和智利的茎柔鱼分开, 而Cu⁶³又可将秘鲁与智利的茎柔鱼分开。分析认为, 秘鲁和智利外海相互之间较高的误判率说明二者可能共同属于“南半球”种群, 而厄瓜多尔由于受不同的海洋环境影响可能属于独立于“南半球”和“北半球”种群之外的另一个种群。研究认为, 茎柔鱼眼睛晶体核心微量元素的差异是判定其地理种群的一种新方法, 为其他头足类种群划分提供了新途径。

关键词: 茎柔鱼; 眼睛晶体; 微量元素; 地理种群; 判别分析

中图分类号: S 917.4

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茎柔鱼(*Dosidicus gigas*), 俗称大赤鱿, 广泛分布于东太平洋阿拉斯加至智利南部的近岸以及大洋水域的海表面至海表面以下1 200 m水层^[1]。其位于东太平洋海洋食物网的中层, 在营养传输上起着关键作用^[2], 一方面它捕食甲壳类、鱼类和头足类等各种生物^[3-5], 另一方面它又是许多大型鱼类、哺乳动物和海鸟的饵料^[6-8]。茎柔鱼资源丰富, 资源量主要受环境和补充量变化

影响^[9-11], 主要渔场位于加利福尼亚湾^[12-13]、哥斯达黎加冷水丘附近^[12,14]以及秘鲁^[14-15]、智利^[14,16-18]和厄瓜多尔^[19-20]的近岸和外海。茎柔鱼种群结构复杂, 厄尔尼诺和拉尼娜现象引起的海洋环境变化可使其改变生物学特性, 例如能量在生长和生殖投入中的分配^[21], 继而改变种群结构。

了解种群结构及其相互关系是掌握海洋生物种群动力学的关键, 同时也为资源的有效管

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理和可持续发展提供基础^[22-23]。头足类传统的种群判别方法有形态学和分子生物学^[24]，近年来基于微量元素的种群判别方法被认为是头足类种群判定方法的有力补充^[24]。海洋生物早期生活史时期形成的硬组织携带的自然标记，记录了其出生地及其附近的环境信息^[22,25]。因此，头足类胚胎期形成的耳石核心区的微量元素可用作判定种群、追溯不同种群的出生地^[26-27]。例如，Ba/Ca一般指示上升流的存在，所以较高的Ba/Ca说明生物体经历了比较强的上升流^[24,28-29]。然而在实际的研究中，鱼类早期生活史时期的耳石很难获取，研究认为可采用形成于这个时期的成鱼的耳石核心区来替代^[27,30-31]。

除了耳石，眼睛晶体也是头足类重要硬组织之一。本研究采用激光剥蚀—电感耦合等离子体质谱法(laser ablation inductively coupled plasma mass spectrometry, LA-ICPMS)测定了东南太平洋厄瓜多尔、秘鲁和智利外海茎柔鱼眼睛晶体核心的微量元素，分析晶体核心的微量元素在各海区之间的差异，探讨其在茎柔鱼地理种群判别中的效果与可行性，期待为其他头足类种群的划分提供新方法，研究结果有助于深入理解东南太平洋茎柔鱼地理种群的连通性，为资源的管理与开发提供科学支撑。

1 材料与方法

1.1 样本采集和处理

中国鱿钓船于2015年和2017年在东南太平洋专属经济区(exclusive economic zone, EEZ)外海79°00'~91°52' W、3°21'~40°00'S(图1)共采集茎柔鱼样本78尾，其中厄瓜多尔外海23尾(84°07'~91°52' W、3°21'~8°26'S，胴长范围210~357 mm，体质量264~1 295 g)，秘鲁外海30尾(79°45'~85°03' W、9°16'~15°22'S，胴长范围226~352 mm，体质量280~1 301g)，智利外海25尾(79°00'~83°00' W、37°06'~40°00'S，胴长范围210~560 mm，体质量151~3 994 g)(表1)。所有样品在船上冷冻保存，

在实验室解冻后进行生物学测定、性别鉴定和性成熟划分。测定胴长精确至1 mm，体质量精确至1 g。采用手术刀在角膜中切口，用镊子取出眼睛晶体，用游标卡尺测量晶体直径，精确至0.01 mm。晶体外部组织和玻璃体材料则用离子水冲洗、人工去除，然后用75%乙醇保存，用于进一步分析。

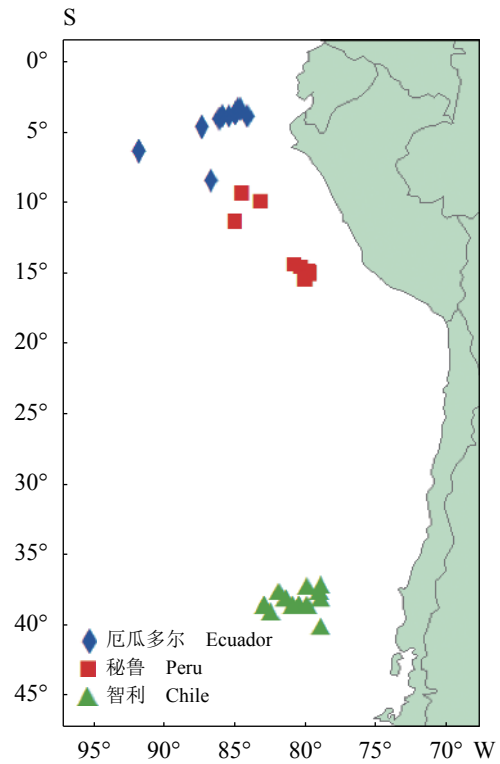


图1 东南太平洋专属经济区外海茎柔鱼采样站点分布
Fig. 1 Sampling locations of *D. gigas* off the EEZ in the Eastern Pacific Ocean

1.2 眼睛晶体的处理

取出保存在离心管中的眼睛晶体，用游标卡尺测定晶体最大直径。由于眼睛是由前后两个部分组成的(图2)，取出后将两者分离，保存体积较大的后半部分(图3-a)，使其平放于塑料模具中(图3-b)，倒入配置好的冷埋树脂溶液进行包

表1 东南太平洋专属经济区外海茎柔鱼样本信息

Tab. 1 Sampling information of *D. gigas* off the EEZ in the Eastern Pacific Ocean

海区 sea areas	采样日期 sampling date	采样地点 sampling locations	样本量/尾 sample size	胴长/mm ML	体质量/g BW
厄瓜多尔 Ecuador	2017年7—8月	84°07'~91°52' W、3°21'~8°26'S	23	210~357	264~1 295
秘鲁 Peru	2015年6—9月	79°45'~85°03' W、9°16'~15°22'S	30	226~352	280~1 301
智利 Chile	2015年11—12月	79°00'~83°00' W、37°06'~40°00'S	25	210~560	151~3 994

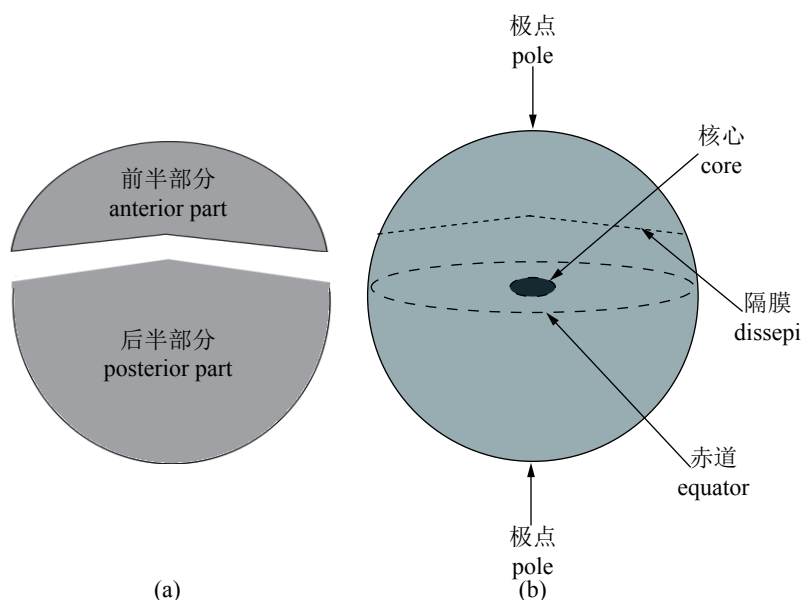


图 2 眼睛晶体示意图

(a) 眼睛晶体前后两部分; (b) 眼睛晶体结构组成

Fig. 2 Diagrams of eye lens of *D. gigas*

(a) the anterior and posterior parts of the eye crystal; (b) the structure of the eye crystal

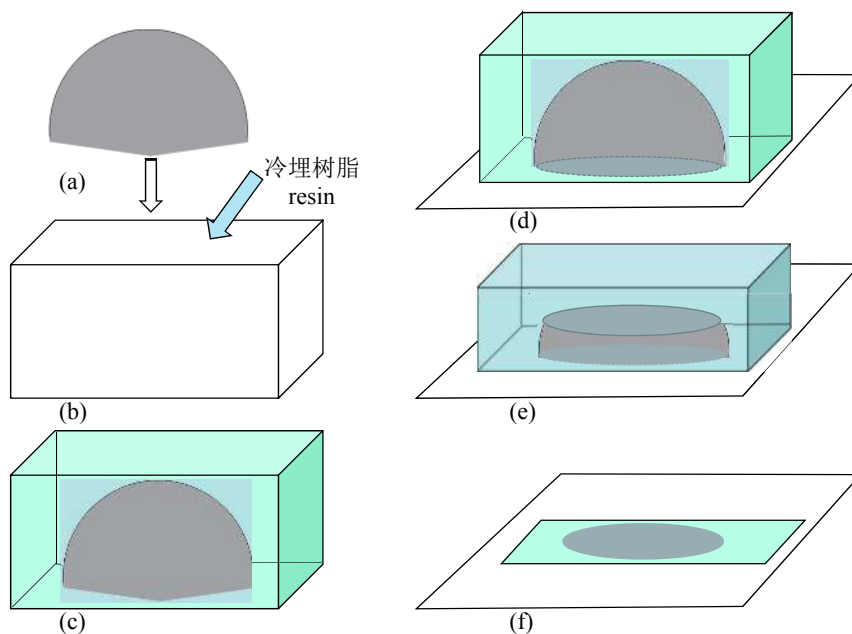


图 3 眼睛晶体切片制作流程图

(a) 已分离的眼睛晶体的后半部分; (b) 塑料磨具; (c) 经过冷埋树脂包埋的眼睛晶体后部; (d)~(f) 切割后的树脂块黏于载玻片上并研磨至赤道面

Fig. 3 Flow chart of eye lens preparation

(a) the posterior part of the separated eye lens; (b) plastic abrasive tools; (c) the back of the eye lens embedded in the resin; (d)~(f) the cut resin block is adhered to the slide and ground to the equatorial surface

埋(图3-c), 在常温避光处放置待其硬化; 将硬化后的树脂块用强力热熔胶黏于载玻片上, 先将底部磨平(图3-d), 然后180°倒转研磨另一面(图3-e),

研磨时先后以120、600、1 200、2 500目水磨砂纸研磨成约0.5 mm的薄片, 最后以0.05 μm氧化铝剂抛光研磨面(图3-f)。

1.3 晶体微量元素的测定

为减少污染物对元素测试带来的干扰, 研磨后的晶体切片在电阻率 $>18\ \Omega$ 的去离子水中清洗5 min并在Class-100层流柱中晾干。在晶体的核心选取1个取样点(图4)。

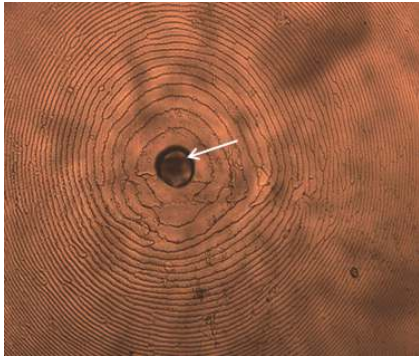


图4 茎柔鱼早期生活史时期眼睛晶体取样点

Fig. 4 Analysis spots in one of ground statolith formed at early ontogenetic phase of *D. gigas*

晶体微量元素含量在武汉上谱分析科技有限责任公司利用LA-ICP-MS完成。详细的仪器参数和分析流程参考Zong等^[32]。GeolasPro激光剥蚀系统由COMPexPro 102 ArF 193 nm准分子激光器和MicroLas光学系统组成, ICP-MS型号为Agilent 7700e。激光剥蚀过程中采用氦气(0.7 L/min)作载气, 氩气(0.8 L/min)为补偿气以调节灵敏度, 二者在进入ICP之前通过一个T型接头混合, 激光剥蚀系统配置有信号平滑装置。本次分析的激光束斑直径和频率分别为44 μm 和5 Hz。微量元素含量处理中采用玻璃标准物质BHVO-2G、BCR-2G、BIR-1G和SRM610进行多外标无内标校正。每个时间分辨分析数据包括大约20~30 s空白信号和50 s样品信号。对分析数据的离线处理(包括对样品和空白信号的选择、仪器灵敏度漂移校正以及元素含量计算)采用软件ICPMSDataCal完成^[32]。

1.4 数据与统计分析

对于浓度大于仪器LA-ICP-MS测定限值的元素采用单因素方差分析(One-Way, ANOVA)和多因素方差分析(multivariate analyses of variance, MANOVA)检验在各海区之间的差异。采用逐步判别分析(stepwise discriminant analysis, SDA)法划分茎柔鱼不同地理种群。利用逐步判别分析的前两个判别函数系数及其均值计算95%椭圆置信区间。运用留一法交叉验证(leave-one-out cross-

validation), 又叫折叠再分类法(jackknife reclassification procedure)检验种群划分的成功率。运用随机检验(randomization test)检查留一法交叉验证所得判别成功率是否由随机误差造成的^[33]。95%椭圆置信区间由R3.4.1软件计算, 其他统计分析采用SPSS 25.0软件完成。

2 结果

2.1 微量元素含量

实验对眼睛晶体的全周期表元素进行了测定, 结果大于仪器检测限的有效元素有钠 Na^{23} 、镁 Mg^{25} 、铝 Al^{27} 、硅 Si^{29} 、磷 P^{31} 、钾 K^{39} 、钙 Ca^{43} 、铜 Cu^{63} 、锌 Zn^{66} 、锶 Sr^{88} 和铅 Pb 等11种(表2), 其中 P^{31} [(326 680 \pm 98 736) $\mu\text{g/g}$]含量最高, 其次为 Ca^{43} [(50 659 \pm 10 9546) $\mu\text{g/g}$]、接下来依次为 Si^{29} [(42 059 \pm 34 625) $\mu\text{g/g}$]、 Na^{23} [(19 033 \pm 32 360) $\mu\text{g/g}$]、 Al^{27} [(14 906 \pm 52 770) $\mu\text{g/g}$]、 K^{39} [(13 432 \pm 23 397) $\mu\text{g/g}$]、 Mg^{25} [(8 954 \pm 8 118) $\mu\text{g/g}$]、 Zn^{66} [(2 360 \pm 1 024) $\mu\text{g/g}$]、 Cu^{63} [(221 \pm 194) $\mu\text{g/g}$]、 Sr^{88} [(84 \pm 105) $\mu\text{g/g}$]和 Pb [(23 \pm 14) $\mu\text{g/g}$]。

按海区分析显示, 厄瓜多尔 P^{31} [(34 2121 \pm 96 021) $\mu\text{g/g}$]含量最高, 其次为 Si^{29} [(51 194 \pm 59 343) $\mu\text{g/g}$]、接下来依次为 Al^{27} [(24 658 \pm 79 871) $\mu\text{g/g}$]、 Ca^{43} [(23 265 \pm 30 825) $\mu\text{g/g}$]、 Na^{23} [(11 744 \pm 18 069) $\mu\text{g/g}$]、 K^{39} [(5 071 \pm 6 474) $\mu\text{g/g}$]、 Zn^{66} [(2 241 \pm 773) $\mu\text{g/g}$]、 Mg^{25} [(1 774 \pm 2 780) $\mu\text{g/g}$]、 Cu^{63} [(228 \pm 229) $\mu\text{g/g}$]、 Pb [(25 \pm 11) $\mu\text{g/g}$]和 Sr^{88} [(18 \pm 20) $\mu\text{g/g}$]; 秘鲁 P^{31} [(28 1544 \pm 122 290) $\mu\text{g/g}$]含量最高, 其次为 Ca^{43} [(94 391 \pm 16 6130) $\mu\text{g/g}$]、接下来依次为 Si^{29} [(39 848 \pm 18 722) $\mu\text{g/g}$]、 Na^{23} [(32 341 \pm 46 490) $\mu\text{g/g}$]、 K^{39} [(25 409 \pm 33 941) $\mu\text{g/g}$]、 Al^{27} [(16 153 \pm 48 713) $\mu\text{g/g}$]、 Mg^{25} [(14 039 \pm 8 885) $\mu\text{g/g}$]、 Zn^{66} [(2 028 \pm 1 134) $\mu\text{g/g}$]、 Sr^{88} [(143 \pm 142) $\mu\text{g/g}$]、 Cu^{63} [(132 \pm 81) $\mu\text{g/g}$]和 Pb [(18 \pm 10) $\mu\text{g/g}$]; 智利 P^{31} [(366 637 \pm 17 798) $\mu\text{g/g}$]含量最高, 其次为 Si^{29} [(36 308 \pm 8 608) $\mu\text{g/g}$]、接下来依次为 Ca^{43} [(23 383 \pm 19 912) $\mu\text{g/g}$]、 Na^{23} [(9 769 \pm 9 328) $\mu\text{g/g}$]、 Mg^{25} [(9 457 \pm 5 193) $\mu\text{g/g}$]、 K^{39} [(6 751 \pm 5 755) $\mu\text{g/g}$]、 Al^{27} [(4 436 \pm 3 445) $\mu\text{g/g}$]、 Zn^{66} [(2 867 \pm 919) $\mu\text{g/g}$]、 Cu^{63} [(322 \pm 210) $\mu\text{g/g}$]、 Sr^{88} [(73 \pm 46) $\mu\text{g/g}$]和 Pb [(27 \pm 18) $\mu\text{g/g}$](表2)。

2.2 微量元素地理区域差异

厄瓜多尔含量前5的微量元素依次为 P^{31} 、 Si^{29} 、 Al^{27} 、 Ca^{43} 和 Na^{23} , 秘鲁依次为 P^{31} 、 Ca^{43} 、 Si^{29} 、 Na^{23} 和 K^{39} , 智利依次为 P^{31} 、 Si^{29} 、 Ca^{43} 、 Na^{23} 和 Mg^{25} ,

表 2 东南太平洋茎柔鱼眼睛晶体微量元素含量
 Tab. 2 Concentration of trace elements in eye lenses of *D. gigas* off the EEZ in the Eastern Pacific Ocean

元素 elements	三海区总体 total	厄瓜多尔 Ecuador	秘鲁 Peru	智利 Chile
钠 Na ²³	19 033±32 360	11 744±18 069 ^a	32 341±46 490 ^b	9 769±9 328 ^a
镁 Mg ²⁵	8 954±8 118	1 774±2 780 ^a	14 039±8 885 ^b	9 457±5 193 ^c
铝 Al ²⁷	14 906±52 770	24 658±79 871 ^a	16 153±48 713 ^a	4 436±3 445 ^a
硅 Si ²⁹	42 059±34 625	51 194±59 343 ^a	39 848±18 722 ^a	36 308±8 608 ^a
磷 P ³¹	326 680±98 736	342 121±96 021 ^{ab}	281 544±122 290 ^a	366 637±17 798 ^b
钾 K ³⁹	13 432±23 397	5 071±6 474 ^a	25 409±33 941 ^b	6 751±5 755 ^a
钙 Ca ⁴³	50 659±109 546	23 265±30 825 ^a	94 391±166 130 ^b	23 383±19 912 ^a
铜 Cu ⁶³	221±194	228±229 ^{ab}	132±81 ^a	322±210 ^b
锌 Zn ⁶⁶	2 360±1 024	2 241±773 ^a	2 028±1 134 ^a	2 867±919 ^b
锶 Sr ⁸⁸	84±105	18±20 ^a	143±142 ^b	73±46 ^c
铅 Pb	23±14	25±11 ^{ab}	18±10 ^a	27±18 ^b

注: 不同字母表示差异性显著 ($P < 0.05$)

Notes: Different letters indicate significant difference ($P < 0.05$)

可以看出除了P³¹在三海区中都为含量最高的元素以外, 其余元素含量高低顺序在三海区之间有一定的变化。ANOVA分析显示, 除了Al²⁷和Si²⁹以外的其余各元素在三海区之间存在显著差异($P < 0.01$, 表3), 然而在这些显著差异的元素中又仅Mg²⁵和Sr⁸⁸在两两海区之间存在显著差异,

表 3 东南太平洋茎柔鱼眼睛晶体核心微量元素
三海区之间方差分析结果

Tab. 3 The result of ANOVA of trace elements in eye lens within 3 areas of *D. gigas* off the EEZ in the

Eastern Pacific Ocean				
	方差平方和 sum of squares	均方差 mean square	P值 P value	
钠 Na ²³	8.681×10 ⁹	4.340×10 ⁹	0.014	
镁 Mg ²⁵	1.968×10 ⁹	9.838×10 ⁸	0.000	
铝 Al ²⁷	4.975×10 ⁹	2.487×10 ⁹	0.415	
硅 Si ²⁹	2.893×10 ⁹	1.446×10 ⁹	0.303	
磷 P ³¹	1.065×10 ¹¹	5.326×10 ¹⁰	0.003	
钾 K ³⁹	7.027×10 ⁹	3.514×10 ⁹	0.001	
钙 Ca ⁴³	9.323×10 ¹⁰	4.662×10 ¹⁰	0.019	
铜 Cu ⁶³	4.977×10 ⁵	2.489×10 ⁵	0.001	
锌 Zn ⁶⁶	1.006×10 ⁷	5.031×10 ⁶	0.007	
锶 Sr ⁸⁸	2.979×10 ⁵	1.035×10 ⁵	0.000	
铅 Pb	1.246×10 ³	6.228×10 ²	0.038	

其他元素至少在某两个海区之间无显著差异。

2.3 逐步判别分析

选取地理差异显著的Na²³、Mg²⁵、P³¹、K³⁹、Ca⁴³、Cu⁶³、Zn⁶⁶、Sr⁸⁸和Pb等9种元素进行逐步判别分析, 结果3个元素(Mg²⁵、Cu⁶³和Zn⁶⁶)最终被用于判别, 标准化判别系数和Wilks' λ 值显示, Mg²⁵和Cu⁶³分别在第一和第二判别函数上贡献了大部分地理差异(表4, 表5)。逐步判别分析结果显示, 利用前两个判别函数可将不同地理区域的茎柔鱼分开, 第1和第2判别函数分别解释75.6%和24.4%的变化率(图5)。交叉验证所得三海区茎柔鱼总体判别成功率为69.2%(随机检验 $P < 0.05$), 其中厄瓜多尔、秘鲁和智利外海分别为87.0%、70.0%和52.0%(表6)。如果将秘鲁与智利外海两海区样本合并后, 再与厄瓜多尔进行判别, 则总体判别成功率上升至89.7%, 厄瓜多尔、秘鲁与智利外海分别为95.7%和87.3%(表7)。

3 讨论

过去的研究显示, 由于耳石中微量元素的富集反映其所生活的水环境的物理和化学特性, 因此可用来示踪头足类的种群、洄游以及栖息环境等生活史过程^[34-39]。一些研究通过耳石整体微量元素的差异来判定鱼类^[40]和头足类种群^[24]。然而有研究质疑, 耳石整体微量元素不能正确

表 4 东太平洋各海区茎柔鱼眼睛晶体核心
微量元素标准化判别系数

Tab. 4 Standardized canonical discriminant functions (DF)
coefficients 1 and 2 for each element used in SDA for
D. gigas among areas

元素 elements	标准化系数1 DF1	标准化系数2 DF2
镁 Mg ²⁵	1.153	0.085
铜 Cu ⁶³	-0.090	0.712
锌 Zn ⁶⁶	0.627	0.580

注: 判别系数绝对值大小表示各元素对各判别系数的相对贡献率
Notes: Coefficients represent the relative contribution of each element to each DF

反映群体信息。例如, 当群体聚集产卵而分散生活时, 就可能错误的将一个单一群体误判为多个群体^[41], 因为耳石微量元素的差异是由不同的生活水环境造成的, 而不是群体本身的不同引起的。与之相比, 形成于生活史早期的硬组织的微量元素更适合用来判定种群和追溯种群起源^[27,34,42-44]。与耳石相似, 眼睛晶体因其代谢惰性使得沉积其中的微量元素信息得以永久保存, 因此已有一些学者将其用于鱼类种群研究^[45-48]。本研究分析了东南太平洋厄瓜多尔、秘鲁和智利外海的茎柔鱼眼睛晶体核心的微量元素在各海区之间的差异, 探讨其在茎柔鱼地理种群判别中的效果与可行性。

胚胎期是头足类出生前的一个发育期, 此时胚胎的表面具有一层保护膜, 它可以阻碍金属元素的吸收^[49]。胚胎靠自身携带的卵黄囊给予营养, 此时组织中的微量元素来源于母体, 而与外界环境无关^[29,43,50]。因此可用来划分头足类种群。例如, Warner等^[27]和Doubleday等^[42]分别通过分析乳光枪乌贼(*Doryteuthis opalescens*)成体的耳石核心以及苍白蛸(*Octopus pallidus*)初孵幼体内骨针的微量元素对它们的地理种群进行了划分。眼睛晶体作为头足类重要硬组织之一, 其核心部分也形成于胚胎期。本实验采用逐步判

别法对不同海区的茎柔鱼眼睛晶体核心的微量元素进行了分析, 结果显示, 晶体核心的差异可用来判别茎柔鱼不同地理群体, 总体判别成功率为69.2%, 该结果比通过耳石核心的微量元素判别效果要好(判别成功率为61.0%^[43]), 但是较利用角质颞形态判别的效果差(判别成功率为89.5%^[51])。Kingsford等^[48]研究发现, 小鳞盾豆娘鱼(*Parma microlepis*)眼睛晶体中的Mn⁵⁵、Sr⁸⁵和Ba¹³⁸含量与耳石中的相当, 但Rb⁸⁵的含量却有很大的差别, 而这种差异来自于栖息环境的差异, 因此Rb⁸⁵可以作为群体划分的指标。本研究发现, 除了Al²⁷和Si²⁹以外的其余各元素在三海区之间均存在极显著差异($P < 0.01$), 而Mg²⁵的差异是判定茎柔鱼不同地理群体的主要指标。然而, 最近也有研究认为眼睛晶体不适用于一些鱼类种群的判别。例如, Pourang等^[47]检测了库图拟鲤(*Rutilus kutum*)的耳石、鳞片、脊椎骨和眼睛晶体等硬组织的微量元素发现, 鳞片和脊椎骨微量元素的种类组成和含量与耳石相当, 因此认为它们在种群鉴定时可以代替耳石, 而眼睛晶体的元素种类组成及含量与耳石差异较大, 不适合种群判定。耳石相当, 因此认为它们在种群鉴定时可以代替耳石, 而眼睛晶体的元素种类组成及含量与耳石差异较大则不适合种群判定。

茎柔鱼广泛分布于东太平洋, 其群体结构复杂, 一般可根据成体的个体大小分为3个主要种群^[6]: 小型群(雌、雄胴长分别为140~340 mm和130~260 mm)主要分布在赤道附近水域, 中型群(雌、雄胴长分别为280~600 mm和240~420 mm)在其栖息范围内都有分布, 大型群(雌性胴长为550~650 mm至1 000~1 200 mm, 雄性胴长为>400 mm)主要分布于东太平洋南北半球高纬度地区。另外一种方法是根据洄游策略将其划分为“南半球”、“北半球”两个地理群体^[52-53], 后面分子遗传学分析证明了这一点^[54-55], 而这种南北分化大约开始于近1万年^[56]前。然而, 同为“南半球”群

表 5 基于眼睛晶体微量元素的逐步判别分析结果

Tab. 5 Summary of stepwise discriminant analysis showing the order of variables to entry for the three areas

判别步数 steps	变量 variable	输入F量 F to enter	Wilks's λ	统计F量 F statistic	自由度1 DF1	自由度2 DF2
1	镁 Mg ²⁵	23.752	0.612	23.752	2	75
2	锌 Zn ⁶⁶	10.388	0.478	16.515	4	148
3	铜 Cu ⁶³	4.209	0.429	12.835	6	146

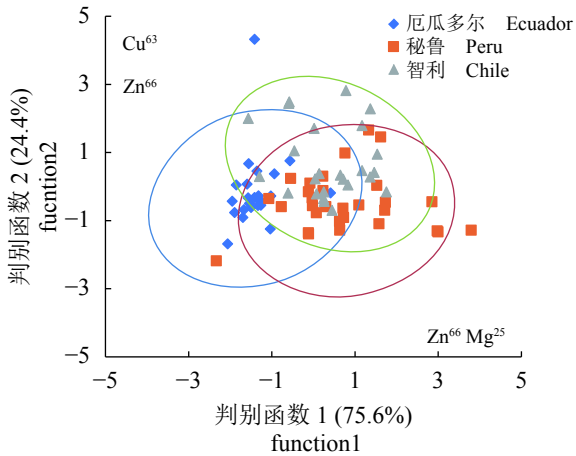


图 5 茎柔鱼眼睛晶体核心微量元素判别分析 (椭圆表示95%置信区间)

Fig. 5 Discriminant function plots of trace elements in the core of eye lenses for *D. gigas* among three areas (Ellipses represent 95% confidence interval around centroids of each group)

表 6 基于三海区眼睛晶体微量元素的逐步判别成功率

Tab. 6 The result of a classification matrix with percentages of correctly classified individuals based on trace element variables of three areas

海区 sea areas	判别成功率/% correct classification rate	被成功判别的样本数/尾 number of corrected classified		
		厄瓜多尔 Ecuador	秘鲁 Peru	智利 Chile
厄瓜多尔 Ecuador	87.0	20	1	2
秘鲁 Peru	70.0	4	21	5
智利 Chile	52.0	3	8	14
总体 total	69.2	27	30	21

表 7 基于两海区眼睛晶体微量元素的逐步判别成功率

Tab. 7 The result of a classification matrix with percentages of correctly classified individuals based on trace element variables of two areas

海区 sea areas	判别成功率/% correct classification rate	被成功判别的样本数/尾 number of corrected classified	
		厄瓜多尔 Ecuador	秘鲁与智利 Peru and Chile
厄瓜多尔 Ecuador	95.7	22	1
秘鲁与智利 Peru and Chile	87.3	7	48
总体 total	89.7	29	49

体的秘鲁和智利海域茎柔鱼遗传分化不明显^[54,57-58]。

逐步判别分析结果显示, 眼睛晶体核心的微量元素可以用来判别茎柔鱼不同地理群体,

厄瓜多尔、秘鲁和智利外海的判别成功率分别为87.0%、70.0%和52.0%。不难看出, 秘鲁和智利外海茎柔鱼的判别成功率明显低于厄瓜多尔外海, 这是因为秘鲁和智利外海各自有相当一部分样本被错判至对方海域所致。秘鲁外海有16.7%的样本被错判至智利外海, 而智利外海有高达56.0%的样本被错判至秘鲁外海。这种误判现象在耳石核心区微量元素中也有发现。采用耳石核心区微量元素来区分哥斯达黎加、秘鲁和智利外海茎柔鱼的地理种群^[43], 结果判别成功率仅为61.0%, 这也是因为秘鲁和智利外海相当高的误判率所致(30.8%的秘鲁样本被错判至智利, 34.8%的智利样本被错判至秘鲁)。而当把秘鲁和智利的样本合并后再与哥斯达黎加的样本进行逐步判别分析发现整体判别成功率提高至84.7%, 本实验合并后整体判别成功率提高至89.7%。因此分析认为, 哥斯达黎加外海的茎柔鱼属于“北半球”种群, 秘鲁和智利外海的茎柔鱼属于“南半球”。本研究的研究结果也证明, 尽管眼睛晶体核心微量元素也能将秘鲁和智利外海的茎柔鱼部分分开, 但是两者相互之间较高的误判率说明它们可能共同属于“南半球”种群。海洋学环境(如温度、盐度和海流)和生物学环境(如产卵场位置和食物可利用率)影响着茎柔鱼的资源量及其分布^[56,59-60]。因此, 东太平洋多变的海洋环境, 尤其盛行的海流驱动着早期幼体的分布, 进而影响茎柔鱼种群结构和分布。如果说哥斯达黎加外海受哥斯达黎加冷水丘和北赤道逆流影响属于“北半球”种群, 秘鲁和智利外海受秘鲁寒流和沿岸上升流的影响属于“南半球”种群^[61-62], 那么厄瓜多尔外海位于南北半球之间的赤道附近, 受赤道流和赤道逆流的影响, 可能属于独立于两者之外的另外一个种群。

4 小结

近些年来, 海洋生物生活史早期的硬组织的生物地球化学信息被广泛用于头种群研究。茎柔鱼广泛分布于东太平洋海域, 种群结构复杂。本研究采用LA-ICP-MS测定了东南太平洋厄瓜多尔、秘鲁和智利外海等三个海区茎柔鱼眼睛晶体核心的微量元素, 根据仪器检测限测得有效元素有Na²³、Mg²⁵、Al²⁷、Si²⁹、P³¹、K³⁹、Ca⁴³、Cu⁶³、Zn⁶⁶、Sr⁸⁸和Pb等11种, 尽管各海域含量前5的元素顺序有所变化, 但是均以P³¹含量最高。

单因素方差分析显示,除了Al²⁷和Si²⁹以外的其余各元素在三海区之间存在极显著差异($P < 0.01$)。逐步判别分析结果显示,眼睛晶体核心的微量元素可以用来判别茎柔鱼不同地理群体,总体判别成功率为69.2%,厄瓜多尔、秘鲁和智利外海的判别成功率分别为87.0%、70.0%和52.0%;Mg²⁵可以用来区分厄瓜多尔与秘鲁和智利的茎柔鱼。如果将秘鲁与智利外海两海区样本合并后,再与厄瓜多尔进行判别,则总体判别成功率上升至89.7%,厄瓜多尔、秘鲁与智利外海分别为95.7%和87.3%。而Cu⁶³又可以将秘鲁与智利的茎柔鱼分开。秘鲁和智利外海相互之间较高的误判率说明两者可能共同属于“南半球”种群,而厄瓜多尔由于受不同的海洋环境影响可能属于独立于“南半球”和“北半球”种群之外的另一个种群。此外值得注意的是,本研究的样本来自于不同年份、不同月份,这对实验结果可能存在的一定影响,然而大洋性采样成本高,操作困难,尽管如此今后仍希望能够通过长时间序列数据的积累尽量减少采样不统一带来的误差。

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Discrimination of geographical population of jumbo flying squid (*Dosidicus gigas*) using the trace elements in the nuclear part of eye lenses

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Abstract: To analyze the difference of trace elements in the nuclear part of eye lenses in different sea areas and test the performance of the microelements in the identification of geographical population of *Dosidicus gigas*, the research samples were taken from China's ocean-going squid fishing boats in Ecuadorian, Peruvian and Chilean exclusive economic zones (EEZs) in the southeast Pacific Ocean in 2015 and 2017. The trace elements of eye lenses were determined to study as the population origin by applying the stepwise discriminant analysis, etc. The results showed that the trace elements in the nuclear part of eye lenses were significantly different among the three regions from the Ecuadorian, Peruvian and Chilean EEZs except for Al²⁷ and Si²⁹. The results of stepwise discrimination showed that the trace elements in the nuclear part of the eye lenses could be used to identify different geographical populations of *D. gigas*, and the overall discrimination success rate was 69.2%. The discrimination success rates of Ecuador, Peru and Chile were 87.0%, 70.0% and 52.0%, respectively. If the samples of the two regions from the Peruvian and Chilean EEZs were combined and then judged with Ecuador, the overall success rate rose to 89.7%, compared with 95.70% and 87.30% off the coast of Ecuador, Peru and Chile, respectively. Mg²⁵ can be used to identify *D. gigas* from Ecuador with Peru and Chile, while Cu⁶³ can identify Peruvian and Chilean. The high rate of mis-classification between Peru and Chile indicated that the two may belong to the "Southern Hemisphere" population, while Ecuador may be independent of the "Southern Hemisphere" and "Northern Hemisphere" populations due to different marine environments.

Key words: *Dosidicus gigas*; eye lenses; trace elements; geographical population; stepwise discriminant analysis

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